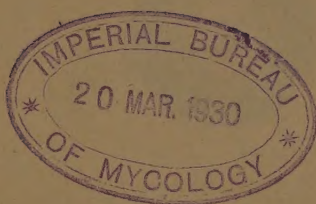




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## EDITORIAL NOTE

**T**HIS research quarterly, or Iowa State College Journal of Science, has been authorized and established for several distinct purposes as follows:

First, to furnish a medium for the prompt publication of the results of research. Experience has shown that many of the technical and scientific journals are at present overcrowded, and it is frequently impossible to secure publication of an article for many months or even a year or more after acceptance.

Second, to give opportunity for the publication of preliminary notes.

Third, to afford space for the publication of some articles of greater length than can usually be accepted by special journals.

Fourth, to give a publication channel to such results of workers on the research staffs as are scarcely long enough to warrant publication as separate bulletins.

Fifth, to provide for the prompt publication of certain of the doctoral dissertations.

The Journal has come into being as the result of the studies and recommendations of a committee acting for the Iowa State College Chapter of the Society of the Sigma Xi, and for the Osborne Research Club. It is to be trusted that in form, content and scope the publication will reach the high standards set by its proponents.





# LINKED INHERITANCE IN TOMATOES

E. W. LINDSTROM.

*From the Genetics Laboratory, Iowa State College.*

Received for publication July 26, 1926.

The tomato (*Lycopersicum esculentum*, Mill.), having the relatively large number of 24 chromosomes, has been investigated but slightly with respect to the hereditary nature of its chromosomal constitution. Preliminary experiments have established that both the independent and linked types of inheritance occur, but the genetical architecture of the germ plasm is not known in any detail.

Only three linkage groups have been determined with any certainty. The first linkage group involves the well-known tall and dwarf plant characters and the smooth and "peach" (pubescent) fruit characters. To this same group apparently belongs a third pair of genes, governing certain differences in fruit shape. Reports by Hedrick and Booth (1907) and MacArthur (1925) have indicated that genetic factors responsible for certain shapes of fruit are linked in inheritance with the tall-dwarf and smooth-pubescent factors. This situation is to be treated in some detail in a subsequent portion of the present report.

To the second linkage group have been assigned the genes *R* and *r*, which control the dominant red and recessive yellow flesh (endocarp) colors of the ripened fruit. This pair of genes has been found to be inherited independently of the *Dd* and *Pp* genes (Lindstrom 1925). Certain hereditary factors for size of fruit also appear to belong to this group (Lindstrom 1926).

In the third linkage group are the genes *Yy* which determine the dominant yellow and recessive colorless conditions of the fruit epidermis. These have proven to be inherited independently of the *Dd* and *Pp* genes, as well as of the *Rr* genes. Factors for size of fruit have also been established in this third linkage group (Lindstrom 1926).

Other linkages have been suggested, but are not thoroly established. One involves green and yellow foliage color and number of locules. Some  $F_2$  data of Price and Drinkard (1908) based only on 24 plants indicate an association of these characters. The situation needs verification. Another case is that reported by Crane (1915) in which general fruit shape (not pyriform) is linked with certain types of inflorescence. Here also the problem is open for further work.

It is the object of this report to present experimental data on the linkage relations within the first group only. These relations will be concerned both with the qualitative genes and the quantitative factors that govern the size and shape of the tomato fruit.

## LINKAGE OF THE DWARF AND PEACH GENES

Dwarfishness in the tomato is controlled by a single gene (*d*) and is recessive to the standard or tall habit of growth (*D*). There is as yet no experimental evidence to show that all dwarf varieties of the tomato are genetically identical. Among other species of plants, dwarfs that are differ-

<sup>1</sup> Paper No. 14 from the Department of Genetics, Iowa State College.

ent genetically are fairly common. Perhaps the same will be found to be true in the tomato. The writer has tested only two dwarf varieties in this connection. Crosses of Dwarf Stone and Dwarf Giant, commercial varieties that resemble each other phenotypically, have invariably given  $F_1$  hybrid progenies that were all dwarfs, indicating genetic similarity.

The "peach" or pubescent type of fruit, characteristic of certain commercial varieties known as peach tomatoes, acts genetically as a single recessive to the familiar smooth sorts of fruit. While the difference between these two kinds of fruits is primarily one of degree of pubescence, and while there is some variation in this respect, especially in hybrid types, there is relatively little difficulty in classifying the fruits into two groups whose hereditary performance can be predicted. If the fruits are observed both in the green and ripe stages, the classification into smooth and peach types becomes reasonably certain.

Hitherto the evidence on the linkage relations of the  $Dd$  (tall-dwarf) and the  $Pp$  (smooth-pubescent) factors has been incomplete, earlier investigations (Lindstrom (1925, MacArthur (1925) ) indicating complete linkage between these two pairs of genes in the  $F_2$  generations of crosses of dwarf smooth by tall peach varieties. Only the following three combinations of these hereditary factors occurred:  $DP$ -tall vine, smooth fruit;  $Dp$ -tall vine, pubescent fruit; and  $dP$ -dwarf vine, smooth fruit. The fourth combination,  $dp$  (dwarf vine, pubescent fruit) has never been reported in  $F_2$  generations, nor is this type of tomato known commercially. It was finally discovered in an  $F_3$  generation of the writer's pedigree cultures in 1924. Since then several selections of the genotype  $dd pp$  have been developed, and have been used to test the linkage relations of the first group of linked genes by means of backcrosses.

Before proceeding with the backcross method, it will be well to present the complete  $F_2$  data of the crosses involving the  $Dd$  and  $Pp$  pairs of genes. The experimental results of  $F_2$  generations from two varietal crosses are arranged in table I.

Only three sorts of fruit emerge in these  $F_2$  generations of table I, giving a total of 180  $DP$  (tall smooth) : 77  $Dp$  (tall peach) : 79  $dP$  (dwarf smooth). This distribution resembles somewhat a 2:1:1 ratio, indicative of a complete linkage or of a triple allelomorphous situation. Such results do not give a critical answer to the exact genetic interrelations of these genes, the difficulty lying, presumably, in the relative fewness of the plants in the test.

The discovery of the fourth combination,  $dp$  (dwarf-pubescent), in an  $F_3$  generation (arising either as a mutation, or what is more probable, as a segregate from a recessive,  $F_2$  crossover gamete in the combination, ( $Dp \cdot dp$ ) paved the way for a definite analysis of the situation. Several  $F_1$  plants ( $Dp \cdot dP$ ) from the dwarf Giant-Yellow Peach cross were fertilized reciprocally with the new, double-recessive

TABLE I.  $F_2$  AND BACKCROSS DATA FROM THE CROSS  $DD pp \times dd PP$  SHOWING LINKAGE RELATIONS

Crosses	DP	Dp	dP	dp	Cross over pct.
$F_2$ generations of					
Dwarf Giant $\times$ Yellow Peach	111	54	52	0	
Dwarf Stone $\times$ Yellow Peach	69	23	27	0	
Total	180	77	79	0	
Backcrosses of					
$F_1$ ( $DG \times YP$ ) $\times$ $pp dd$	2	60	55	2	3.4
$F_1$ ( $DG \times YP$ ) $\times$ $pp DD$ and then progeny tested	4	44	71	0	3.4
Total	6	104	126	2	3.4

type, *dd pp*. The results obtained in the progenies from such backcrosses are summarized in table I.

These critical backcross progenies reveal the fact that there is an extremely close but not complete linkage between the *D* and *P* genes. All four classes or combinations appear, but the non-parental or crossover types (*DP* and *dp*) are exceedingly rare. Thus from a total of 119 individual plants in the backcross, only two *DP* plants and two *dp* plants<sup>1</sup> were observed. This total of four non-parental types gives 3.4 percent of crossing over between *D* and *P*.

This explains why the double-recessive combination had not appeared in the *F*<sub>2</sub> progenies under ordinary experimental conditions. With such infrequent crossing over, one would have to grow nearly 4,000 *F*<sub>2</sub> plants to obtain a single double recessive combination, theoretically. To be reasonably certain of obtaining one such plant, at least 10,000 *F*<sub>2</sub> plants would be required.

Before the advent of the double-recessive type it was decided to test the *DP* situation in another manner. The *F*<sub>1</sub> plants (*Dp . dP*) of the original cross were pollinated with the Yellow Peach (*DD pp*) variety, no peach variety that was dwarf being then available. This cross is essentially a back-cross involving the two pairs of genes in question, but necessitates growing the seedling progenies from the backcross plants for a test of their *DD* or *Dd* genotypes. If *Dd* and *Pp* are linked, the ratio of the resulting progenies should give a measure of the intensity of the linkage. The backcross genotypes and their genetic behavior in the seedling progenies would be as follows:

<i>F</i> <sub>1</sub> gametes	Yellow Peach gametes	Backcross genotypes	Backcross fruits	Progeny behavior
<i>Dp</i> —parental	<i>Dp</i>	<i>DD pp</i>	Peach	Homozygous tall
<i>dP</i> —parental	<i>Dp</i>	<i>Dd Pp</i>	Smooth	Heterozygous
<i>DP</i> —crossover	<i>Dp</i>	<i>DD Pp</i>	Smooth	Homozygous tall
<i>dp</i> —crossover	<i>Dp</i>	<i>Dd pp</i>	Peach	Heterozygous

By growing the seedling progenies, the ratio of the four backcross genotypes could be ascertained. The data for these also appear in table I.

As in the preceding backcross the parental combinations (in this case *Dp . Dp* and *dP . Dp*) are in the great majority. Only four recombinations or crossover classes occurred in a total of 119 types. This gives 3.4 percent crossing over between *D* and *P*, a determination that happens to be identical with the one obtained in the more direct manner. Accordingly it is apparent that the *D* and *P* factors are not completely linked in inheritance, but show a small degree of crossing over.

#### LINKAGE OF THE PEACH AND SHAPE CHARACTERS

Belonging to the first linkage group is a third pair of genetic factors involved in determining the shape of the tomato fruit. Ordinarily genetic factors affecting shape are relatively complex in nature, and are not subject

<sup>1</sup> One of these crossover plants was peculiar in that it apparently had the tall or standard habit of growth but it was distinctly rugose and dark green like a typical dwarf. However its progeny bred true for the dwarf habit.



to a simple analysis on the basis of a single pair of genes. In the tomato, however, there is apparently an exception to this general rule, similar perhaps to the case in the squash in which Sinnott (1922) demonstrated that the round and disk-shaped types of fruit differ genetically by only one major pair of genes. Warren (1924) recently reported cases in the tomato in which general fruit shape is due primarily to two pairs of complementary genes.

The occurrence of a linkage that involved a shape factor and a member of the first linkage group was indicated by some early work of Hedrick and Booth (1907). Jones (1917) pointed out that their data suggested a linkage between the *Dd* genes and a pear shaped factor; and he calculated that there was approximately 20 percent crossing over between them. MacArthur (1925) verified this linkage and also presented some  $F_2$  data showing linkage between this same shape factor and the peach gene. He interpreted his data as showing 17 percent crossing over between *D* and the pear shape factor (*pr*), and 16 percent between *P* and *Pr*.

While no one has as yet proven that the pear shape of tomato fruits is dependent upon a single recessive gene, there is good circumstantial evidence to this effect, arising entirely from  $F_2$  data. The present report affords additional evidence of this relation, using quantitative means of describing the shape of the tomato fruits.

Whereas the previous investigations of this case have dealt with the relations of the pear shape as contrasted with the round or oblate types, the writer has found that the situation is more general, since the same relations hold with the ovate or plum shape. In other words, the shape factor concerned in the experiments is not limited to the pyriform type with its constricted "neck" of the fruit. Undoubtedly there is a series of multiple allelomorphs concerned in these various shapes of the fruit. Accordingly it seems preferable to put the emphasis on the general shape of the fruit as measured by the ratio of the equatorial to the polar diameter (stem to blossom end). For this purpose the shape index of the tomato fruit has been devised. This is given by the following ratio:

$$\text{Shape index} = \frac{\text{equatorial diameter}}{\text{polar diameter}}$$

A round tomato has an index of 1.00; an oblate or flat type, approximately 1.20 or more; and a plum shaped fruit, 0.75.

The varietal cross which first gave evidence of a linkage between *P* and a shape factor was the Red Peach by Yellow Plum. By means of caliper readings to the nearest millimeter, five to eight fruits from each plant were measured for their polar and equatorial (taken half way between the stem and blossom end) diameters. The Red Peach variety gave a mean shape index of 1.14, whereas the Yellow Plum value was 0.76. The  $F_1$  fruits were practically round, the index being 0.98. There was a slight tendency for these fruits to be wedge-shaped, being somewhat broader at the stem end.

An  $F_2$  generation of 61 plants grown in the greenhouse under favorable conditions for such an experiment showed 48 smooth and 13 peach types. It was at once evident that the  $F_2$  peach fruits were all round or

TABLE II. FREQUENCY DISTRIBUTION OF SHAPE INDEX OF TOMATO FRUITS SHOWING LINKAGE WITH THE  $Pp$  GENES

Shape index	Red Peach x Yellow Plum $F_2$ generation		Red Peach x Golden Fig $F_2$ generation	
	P	p	P	p
.66-.60			1	
.61-.65			4	
.65-.70	2		4	
.71-.75	3		3	
.76-.80	2		4	
.81-.85	6		5	
.86-.90	1		7	
.91-.95	3		8	0
.96-1.00	16		6	1
1.01-1.05	6		3	3
1.06-1.10	7	3	5	1
1.11-1.15	2	4		1
1.16-1.20		6		3
1.21-1.25				1
Total	48	13	50	10

oblate, none were ovate or plum shaped. The shape indices for this  $F_2$  generation have been listed in table II.

Measured quantitatively in this manner, it is apparent that there is a radical difference in shape between the smooth and the pubescent  $F_2$  fruits. There was a distinct tendency for the peach fruits to carry the same shape as their grandparental type, the Red Peach variety. None were ovate; nor were there any round fruits among these peach types. With such a small number of plants involved, it does not seem desirable to attempt any calculation of the degree of linkage. Using the  $Pr pr$  genes as factors for the oblate-ovate

shapes and using the class 0.86—0.90 as the dividing line, the frequency distribution in table II might be grouped as follows.  $34 P Pr : 14 P pr : 13 p Pr : 0 p pr$ . This would suggest a fairly close linkage between the two pairs of genes.

Another cross of similar nature (Red Peach x Golden Fig) gave comparable results which also appear in table II. The Golden Fig variety in this case carries more of a pyriform shape than does the Yellow Plum, and this is reflected in the smaller indices of this  $F_2$  generation. In fact, many of the  $F_2$  fruits were distinctly pear shaped in this cross. There is the same evidence of linkage here, in that none of the  $F_2$  peach sorts exhibit the ovate or pear shape, with the possible exception of one plant that had a shape index of 0.95. This might be interpreted as a crossover combination but there could be no certainty concerning such an interpretation. Grouping the fruits as in the preceding cross, a distribution can be arranged as follows:  $38 P Pr : 12 P pr : 10 p Pr : 0 p pr$ . Again there is clear evidence of a close linkage, but the numbers are too small to be useful in determining the degree of the linkage. It is doubtful if  $F_2$  data in such cases will ever give an exact determination of the crossover percentage. A backcross is needed, but first a good ovate, peach type of plant is required for the double recessive condition. This will have to be searched for in  $F_2$  generations of large numbers, or in  $F_3$  progenies from peach-type,  $F_2$  plants, since there are no commercial varieties of this nature available.

These two second generation progenies afford additional evidence then that a third pair of genes resides on the first chromosome of the tomato. Were it not for the fact that MacArthur (1925), in a preliminary note, had symbolized this pair of shape genes by  $Pr pr$  (from pear), it would seem more appropriate to generalize them as oblate-ovate ( $Oo$ ) genes, since they are apparently not restricted to the pyriform versus the round or flat shapes. Apparently these genes also have an influence on the locule number of the tomato fruit. All plum or pear shaped fruits in the  $F_2$  generations, at least those having shape indices of 0.90 or less, were inclined to be two loculed, whereas many of the round or peach types had a high proportion of three-loculed fruits, similar to their grandparental type, the Red Peach.

RELATIONS BETWEEN THE *Pp* GENES AND FRUIT SIZE (WEIGHT)

Having noted that one chromosome pair in the tomato carries three pairs of linked genes, it becomes highly interesting to determine if any general size factors reside thereon. In a previous publication (1926) the writer demonstrated beyond reasonable doubt that genetic linkage exists between certain fruit size factors and the *Rr* flesh color genes of the second linkage group, as well as the *Yy* skin color genes of the third group. In other words, the second and third chromosomes of the tomato carry general size factors as well as color factors.

A large number of varietal crosses involving the first chromosome genes and different sizes of fruit have been investigated. The technique of this work was described in an earlier paper (Lindstrom (1926)). The general idea in this experiment was to cross pure varieties in combinations as follows:

1. Large peach x small smooth
2. Large smooth x small peach

Given such combinations, it is possible to determine whether there is any genetic association between size and the *Pp* factors in hybrid generations. If none exists, the average fruit size in  $F_2$  for example, should be identical for the smooth or the pubescent fruits.

The general results with the smooth-peach characters are somewhat conflicting, a situation that was not encountered in similar experiments with the *Rr* and *Yy* color genes. In table III are five sets of varietal crosses in which the larger variety contributed the smooth (non-peach) type of fruit. Average weights of fruit of the  $F_2$  and backcross generations are given for a sufficient number of plants to carry some significance. In three of the sets (Bonny Best x Yellow Peach, Dwarf Giant x Yellow Peach, Dwarf Stone x Yellow Peach) there is a consistent and really astonishing

TABLE III. FIVE CROSSES OF *PP* x *pp* IN WHICH THE SMOOTH PARENT CONTRIBUTED THE LARGER SIZE OF FRUIT.

Crosses	Grown	<i>P</i>		Difference and probable error	No. plants	
		Smooth fruit Weight in grams	Peach fruit Weight in grams		<i>P</i>	<i>p</i>
Bonny Best	Field 1924	237.6			10	0
Yellow Peach	Field 1924		51.0		0	10
$F_1$ generation	Field 1924	97.6			20	0
$F_2$ generation	Field 1924	88.9 $\pm$ 1.3	94.6 $\pm$ 2.4	5.7 $\pm$ 2.7	108	40
Backcross						
$F_1$ x Yellow Peach	Field 1924	68.4 $\pm$ 1.1	73.3 $\pm$ 1.1	4.9 $\pm$ 1.6	90	90
Duplicated	Greenhouse 1925	34.9 $\pm$ 0.7	41.4 $\pm$ 0.8	6.5 $\pm$ 1.1	78	42
Dwarf Giant	Field 1924	174.8			9	0
Yellow Peach	Field 1924		51.0		0	10
$F_1$ generation	Field 1924	101.8			10	0
$F_2$ generation	Field 1924	82.5 $\pm$ 1.9	99.2 $\pm$ 2.3	16.7 $\pm$ 3.0	80	40
$F_2$ duplicated	Greenhouse 1925	39.3 $\pm$ 1.0	45.9 $\pm$ 1.5	6.6 $\pm$ 1.9	76	14
Backcross						
$F_1$ x <i>pp</i> dd	Greenhouse 1925	34.7 $\pm$ 0.6	45.9 $\pm$ 0.7	11.2 $\pm$ 0.9	57	62
Dwarf Stone	Greenhouse 1926	89.0			5	0
Yellow Peach	Greenhouse 1926	49.5			10	0
$F_1$ generation	Greenhouse 1926	46.9 $\pm$ 0.7	54.3 $\pm$ 1.4	7.4 $\pm$ 1.5	96	23
$F_2$ generation						
John Baer	Field 1925	119.1			5	0
Yellow Peach	Field 1925		55.6		0	8
$F_2$ generation	Field 1925	74.5 $\pm$ 1.3	72.9 $\pm$ 0.5	1.6 $\pm$ 1.4	103	42
Golden Beauty	Field 1924	118.1			10	0
Red Peach	Field 1924		62.0		0	5
$F_2$ generation	Field 1924	85.9 $\pm$ 2.6	82.2 $\pm$ 1.8	3.7 $\pm$ 3.2	41	9



increase in size (weight) of the peach type of plants in both the  $F_2$  and backcross generations, altho the original peach parent in all cases contributed the smaller size (weight). This state of affairs was not found in two other crosses (John Baer x Yellow Peach and Golden Beauty x Red Peach). Here the smooth  $F_2$  plants carried the larger fruits, altho in these crosses the differences in fruit weight are small and not significant from a mathematical standpoint. Apparently there are differences in behavior traceable to certain varietal combinations.

In the three sets of crosses first mentioned there is no question as to the fact that the peach types emerge from the cross with a markedly greater size. This is true both in the  $F_2$  and in the backcross progenies, since in each of the three crosses, the differences in size between the smooth and peach sorts are statistically significant. Why the peach types are thus larger is uncertain, but there are two general possibilities in explanation, one genetical, the other physiological. The results may be explained genetically by assuming that a linkage occurs between the peach factor and a size factor for larger fruit carried in the smaller Yellow Peach variety, and that the other parents of these crosses (Bonny Best, Dwarf Giant and Dwarf Stone) contributed the allelomorphic, linked factors for smooth fruit and smaller size. In the last two crosses in which the smooth type  $F_2$  plants bore the larger fruits, it may be assumed that the larger smooth, parental varieties in this case (John Baer and Golden Beauty) carried size factors on the first chromosome of greater influence than those of the Yellow Peach variety.

The physiological explanation for the increased size of the peach fruits may rest on the fact that the peach type of plants mature their fruit considerably more slowly than the smooth-fruited type. Accordingly, the longer period of ripening may be a factor in producing larger fruits. This, of course, does not explain why the same situation does not hold for the John Baer x Yellow Peach, or the Golden Beauty x Red Peach crosses. For the time being, the real explanation must be withheld until the case is checked with other combinations of varietal crosses.

In table IV are listed the results of crosses involving the same  $Pp$  genes, but with the peach parent contributing the larger size. There is beautiful consistency in all three crosses of this table in that the peach type  $F_2$  fruits are always significantly larger than the smooth  $F_2$  fruits. In all

TABLE IV. THREE CROSSES OF  $PP \times pp$  IN WHICH THE SMOOTH PARENT CONTRIBUTED THE SMALLER SIZE OF FRUIT.

Crosses	Grown	P		Difference and probable error	No. plants	
		Smooth fruit Weight in grams	Peach fruit Weight in grams		P	p
Yellow Peach	Greenhouse 1925		34.4		0	12
Red Cherry	Greenhouse 1925	5.8			6	9
$F_1$ generation	Greenhouse 1925	9.8			12	0
$F_2$ generation	Greenhouse 1925	$10.5 \pm 0.1$	$15.3 \pm 0.3$	$4.8 \pm 0.5$	105	42
$F_2$ duplicated	Field 1925	$14.9 \pm 0.3$	$21.4 \pm 0.8$	$6.5 \pm 0.8$	68	21
Red Peach	Greenhouse 1925		35.0		0	6
Yellow Plum	Greenhouse 1925	13.0			5	0
$F_1$ generation	Greenhouse 1925	24.8			10	0
$F_2$ generation	Greenhouse 1925	$21.2 \pm 0.4$	$28.5 \pm 0.6$	$7.3 \pm 0.7$	48	13
Red Peach	Field 1925		62.0		0	5
Golden Fig					1	0
$F_2$ generation <sup>1</sup>	Field 1925	$23.6 \pm 0.6$	$39.4 \pm 2.0$	$15.8 \pm 2.1$	50	10

<sup>1</sup> The  $F_1$  seed of this cross was given to the author by Dr. C. E. Myers, Pennsylvania State College.

TABLE V. THREE CROSSES OF  $DD \times dd$  IN WHICH THE TALL PARENT CONTRIBUTED THE LARGER SIZE OF FRUIT.

Crosses	Grown	D Tall Weight in grams	d Dwarf Weight in grams	Difference and prob- able error	No. plants	
					D	d
Dwarf Giant	Field 1924		174.8		0	9
Yellow Peach	Field 1924	51.0			10	0
$F_1$ generation	Field 1924	101.8			10	0
$F_2$ generation	Field 1924	$91.9 \pm 5.9$	$76.1 \pm 2.1$	$15.8 \pm 6.3$	91	29
$F_2$ duplicated	Greenhouse 1925	40.4	40.2		66	23
$F_1 \times pp \ dd$	Greenhouse 1925	$45.9 \pm 0.7$	$34.6 \pm 0.6$	$11.3 \pm 0.9$	62	57
Dwarf Stone	Greenhouses 1925		89.0		0	5
Yellow Peach						
$F_1$ generation	Greenhouse 1925	49.9			10	0
$F_2$ generation	Greenhouse 1925	$48.7 \pm 0.7$	$47.1 \pm 1.2$	$1.6 \pm 1.5$	92	27
Dwarf Giant						
Yellow Cherry						
$F_2$ generation	Greenhouse 1925	$13.7 \pm 0.4$	$16.2 \pm 0.6$	$2.5 \pm 0.7$	66	11

three crosses the differences between the two sorts of  $F_2$  fruits are statistically significant. This, of course, harmonizes with the genetic explanation on the basis of linkage between the  $Pp$  genes and size factors borne on the same chromosome. However, in all of these crosses the larger peach variety was also the later in maturity or time of ripening of the fruit.

#### RELATION OF THE $Dd$ GENES AND FRUIT SIZE (WEIGHT)

Knowing that the  $Dd$  genes are in the same linkage group with the  $Pp$  genes just discussed, it becomes highly interesting to determine the relations between these tall-dwarf genes and size of fruit. Unfortunately, all commercial dwarf varieties of tomatoes are large fruited; at least the writer has not seen a small-fruited dwarf variety. Accordingly, the crosses to test the situation can only be made in one direction. In table V are listed three sets of crosses in which a large-fruited dwarf variety was crossed with a small-fruited standard or tall type.

In two of the three crosses of table V there is a distinct tendency for the dwarf  $F_2$  and backcross plants to bear relatively smaller fruits than the tall plants, despite the fact that the grandparental dwarf variety had much larger fruit than the tall variety. In these two crosses, however, the peach character was also involved, and, because of the close linkage between the  $D$  and  $P$  genes, it is to be expected that the results will be affected by the relation discovered between the  $Pp$  genes and size.

Before commenting on the interpretation of the results in these crosses, it is well to direct attention momentarily to the third cross (Dwarf Giant  $\times$  Yellow Cherry), which does not involve the peach character. In this case there is a noticeable difference in size between the fruits of the tall and the dwarf  $F_2$  plants in favor of the latter. Although this difference is more than three times its probable error the number of plants is really too small to prove anything, but nevertheless there is some indication of a genetic linkage between size and the  $Dd$  genes, in which the large dwarf variety

TABLE VI. SHOWING FRUIT WEIGHT IN RELATION TO VARIOUS COMBINATIONS OF THE  $PpDd$  GENES.

Crosses	Fruit wt. in grams			
	DP	Dp	dP	dp
Dwarf Giant $\times$ Yellow Peach				
$F_2$ generation	86.2	99.2	76.1	
Duplicate in Gr'house	38.9	45.9	40.2	
Backcross of $F_1 \times pp \ dd$ selection	41.5	46.1	34.5	40.0
Dwarf Stone $\times$ Yellow Peach				
$F_2$ generation	46.8	54.3	47.1	

TABLE VII. SHOWING CORRELATION BETWEEN SHAPE OF FRUIT AND SIZE (WEIGHT) IN THE CROSS RED PEACH X YELLOW PLUM.

Shape index	P		p	
	No.	Mean fruit weight grams	No.	Mean fruit weight grams
.66-.70	2	15		
.71-.75	3	16		
.76-.80	2	16		
.81-.85	6	18		
.86-.90	1	21		
.91-.95	3	20		
.96-1.00	16	23		
1.01-1.05	6	25	3	25
1.06-1.10	7	21	4	31
1.11-1.15	2	28	6	28
Mean		21.2		28.2

may be considered as containing a first chromosome that carries both the *d* gene and a size factor for large fruit.

Because of the very close linkage of the *D* and *P* genes and particularly because of the peculiar relations of the *Pp* genes with respect to size of fruit (tables III and IV), it is really essential that the interrelations of both these genes and size of fruit be considered. In table VI, the  $F_2$  and backcross progenies of two varietal crosses have been arranged so as to show the influence of the various combinations of the *Dd Pp* genes on the size of fruit. This table gives a good picture

of the influence of the *p* gene for larger size and the corresponding relation of the *d* gene for smaller size. It suggests that the explanation behind this phenomenon may very well be the existence of a size factor for larger fruit situated rather closely to the *p* gene in the Yellow Peach variety. It will be interesting to learn the experiences of other investigators who have used this variety in their crosses.

Having noted that the peach gene (*p*) is closely associated with a shape gene (*pr*), and also size (*weight*) of fruit, the relation between the shape of the fruit and its size becomes of some interest. The  $F_2$  data of the cross of the Red Peach (medium size) by the Yellow Plum (small) varieties afford some definite results. These are arranged in table VII. In such a cross as this there is excellent correlation between shape and weight, the ovate types showing the smaller weight and the round or oblate fruits being relatively heavier. The actual correlation between shape index and weight in this cross is  $r = .71 \pm .04$ , a very high value. For the Red Peach-Golden Fig cross, the correlation of the same variables gives a coefficient of  $.66 \pm .05$ . Presumably these high correlations are due somewhat to the linkage between the *Pp*, *Pr pr* and size genes noted above, although some of the correlation is obviously inherent in the physical relation of shape and weight.

Experiments having proven that the *d* and *p* genes are linked, as well as the *p* and *pr* genes, it is of course evident that the *d* and *pr* factors must also show linked inheritance. This fact was indicated in the earlier work of Hedrick and Booth (1907) and later by MacArthur (1925). Data from an  $F_2$  generation of the former investigators show 20 percent crossing over between the *d* and *pr* genes, whereas the  $F_2$  determinations of the latter investigator give 17 percent. Inasmuch as the effects of the *pr* genes are not so easily differentiated from those of its dominant allelomorph, because of the semi-quantitative nature of these genes, calculations from  $F_2$  data are apt to be inaccurate. The backcross test is the surest means of obtaining accurate results, but, since there are no ovate, dwarf varieties obtainable, this is not easily done. The writer has resorted to crossing the Yellow Plum variety (tall) on the  $F_1$  plants of dwarf oblate x tall ovate types. This necessitates growing the seedling progenies from the backcrossed plants. The results of this test must be reserved for a future report.

For a real test of the first chromosome linkage group a triple recessive type is needed. This will be a dwarf, peach, ovate sort (*dd pp pr pr*). The writer has made crosses to obtain this type, but it will require large numbers and several years of time.

As a result of these experiments with the first linkage group of the tomato, one cannot help but receive the impression that this first chromosome is not only an important one in the heredity of this species, but also that there is, relatively speaking, little crossing over taking place. Evidence for the latter deduction arises particularly from the close linkage of the *D* and *P* genes and also from the apparently close association of the *D* and *P* genes with general fruit size.

#### SUMMARY

In the first linkage group of tomato the genes *Dd* (tall-dwarf) were found to be very closely linked with the genes *Pp* (smooth-peach or pubescent). The discovery of a double recessive *dd pp* type of fruit, hitherto unknown, provided the means for a backcross test. This resulted in proving that these genes are not completely linked, but that there is approximately 3.4 percent crossing over between them.

Belonging to this same linkage group by virtue of linkage with the *Pp* genes, is a third pair of genes that affect the shape of the tomato fruit. Circumstantial evidence indicates that the ovate or pear shape acts as a simple recessive to the round or oblate type of fruit. By means of a shape index (ratio of equatorial to polar diameter) it is shown that a fairly close linkage occurs in  $F_2$  generations between these shape factors and the *Pp* genes.

With respect to the occurrence of general fruit-size factors on the first chromosome of the tomato, some unusual relations were discovered. In several crosses involving the *Pp* genes and various sizes of fruits, it was found that the peach or pubescent (*p*)  $F_2$  and backcross fruits averaged ten percent greater size than the smooth (*P*) types, despite the fact that the original smooth-fruited grandparent had much the larger fruit. In other crosses the reverse situation held, but not to such a significant degree. In cases where the grandparental variety was of the peach sort and of greater size than the smooth, grandparental variety, the peach type  $F_2$  fruits were always consistently larger than the smooth sorts. The size differences were highly significant from the statistical viewpoint.

In general, the same relations obtained when the *Dd* genes were tested against size. Because of the close linkage between the *D* and *P* genes, this interrelation of qualitative and quantitative (size) factors is extremely suggestive of a genetic rather than a physiological agency operating to link size with fruit and plant characters. The results may be explained genetically on the basis that the Yellow Peach variety carries on its first chromosome a factor for relatively large size in addition to the other known genes, *p*, *Pr* and *D*.

These investigations present further evidence in verification of the theory that size characters are transmitted essentially by the same mechanism of heredity which controls the inheritance of the simpler color characters.



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# TAXONOMIC STUDIES ON SOIL FUNGI\*

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WITH the accumulation of considerable evidence that the filamentous fungi play an important role in soil fertility, particularly in the decomposition of organic matter in the soil, there has come an increased interest in taxonomic studies of these organisms. Previous studies of soil fungi have been confined largely to a study of the physiological functions of these microorganisms, with much less attention being devoted to the species which make up the soil flora. The present work was undertaken for the purpose of supplying information which will be an aid in identifying the fungi occurring commonly in the soil. While it was impossible to identify every organism isolated, detailed studies were made of those forms which occurred regularly in the soils examined.

## REVIEW OF LITERATURE

The first study of soil fungi was made by Adametz (1886), who reported the isolation of *Penicillium glaucum*, *Aspergillus niger*, *Mucor mucedo*, *M. racemosus*, *Monilia candida*, *Oidium lactis*, and several unidentified yeasts, in addition to a number of bacteria, from a sandy soil and a loam soil. Following this work nothing of taxonomic importance concerning soil fungi appeared until 1902, when Oudemans and Koning (1902) reported the isolation of 45 species from the soil, thereby giving the first definite information as to the organisms which constitute the fungous flora of soils.

Later Jensen (1912) worked with cultivated soils in New York, from which he isolated 35 species belonging to several of the common genera. He also assembled the descriptions of fungi which had been reported previously from the soil. Dale (1912), (1914) studied five soils, from which she isolated more than 40 species.

The most extensive study of the soil fungus flora yet attempted was made in 1916 by Waksman (1916), who concluded from his studies of soils from Oregon, California and New Jersey that *Aspergillus*, *Mucor*, *Penicillium* and *Trichoderma* are the most common soil genera. In a later study (1917) he found the same genera predominating in soils which he obtained from Alaska, Canada, Hawaii and Porto Rico, as well as from various points of the United States. Other studies of soil fungi include those of Werkenthin (1916) in Texas, Pratt (1918) in Idaho, Taylor (1917) and Rathbun (1918) in Rhode Island, Takahashi (1919) in Japan, and the writer (1923) in Iowa.

It is interesting to note that in all of these studies, which included soils from many different points in this country as well as from those abroad, many of the same species were found in nearly every instance.

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Members of the two genera, *Penicillium* and *Aspergillus*, were generally found to be predominant, while such forms as *Trichoderma koningi*, *Cladosporium herbarum* and various *Mucors* occurred with considerable regularity. These results support the idea that there is a constant and more or less definite fungous flora of soils, and tend to disprove the older conception of soil fungi as accidental and transitory soil inhabitants.

A complete list of the fungi which have been isolated from the soil will be found in the appendix.

## EXPERIMENTAL

### METHODS

Fungi for identification were isolated from soils of 11 plots on the Agronomy Farm of the Iowa Agricultural Experiment Station. The soils are well adapted for a study of this nature, since they have been under a definite system of cropping and fertilizer treatment for ten years. Four plots represent a livestock system of farming, four a grain system, and three are checks. The fact that six of the plots are on the Carrington loam soil type and five on the Clarion loam, both important types, should make them representative of similar soil conditions thruout the state of Iowa. The fertilizer treatments are as follows:

Plot No.	Treatment
0	Check
1	Manure
2	Manure, lime
3	Manure, lime, rock phosphate
4	Manure, lime, acid phosphate
5	Check
6	Crop residues
7	Crop residues, lime
8	Crop residues, lime, rock phosphate
9	Crop residues, lime, acid phosphae
10	Check

Soil samples for examination were taken monthly, except during the summer, over a period of two years. Dilutions were made and plates poured according to the recommendations of Waksman (1922), using Waksman and Fred's (1922) synthetic acid agar. The plates were incubated at 25° C. for seven days and transfers of the organisms developing were made to agar slants. It was often necessary to purify cultures obtained in this manner.

### CULTURE MEDIA

Czapek's synthetic agar was used as a standard medium for the study of the general habit and appearance of the colony, as well as for detailed morphological characteristics, such as measurements of the various elements of the conidial fructifications. Unless otherwise stated, all measurements reported in the descriptions of species were made from 10 to 12 day old cultures grown on this medium.

Bean agar containing one percent dextrose was used in studying colony characters and also for carrying stock cultures. For all ordinary identification work these two media were sufficient. When following Thom's key (1910) to the *Penicillia*, 15 percent gelatin in water was used for separating



certain members of the group. Gelatin was not particularly helpful in studying other groups. Cooked rice, sterilized milk, and Dox solution were also employed, but they cannot be recommended for ordinary identification work. They may sometimes be used as aids in separating closely related forms.

#### DIFFICULTIES IN THE IDENTIFICATION OF SOIL FUNGI

It is not always possible to identify with certainty all of the fungi which are isolated from the soil. In many instances the published descriptions do not contain sufficient information to enable one to identify the fungus with reasonable accuracy. The older descriptions are in most cases based upon the characteristics of the organism as it occurs in nature, while the soil mycologist must work with cultures on artificial media. It may readily be expected that fungi which are native to the soil will show certain morphological and physiological differences from those isolated from other sources, even though they are representatives of the same species.

In identifying soil fungi, therefore, it is necessary to place a liberal interpretation upon what constitutes a species. Slight variations from the limits of measurement and other morphological characters reported in published descriptions should not be considered as justification for the erection of new species. If the limits of a species are too closely defined, it will be necessary to create many new species in order to classify all of the fungi isolated from the soil. This, of course, should be avoided whenever possible.

#### THE VALUE OF PHYSIOLOGICAL CHARACTERS IN IDENTIFICATION

While morphology is recognized as the primary basis for species differentiation among the fungi, a few attempts have been made to differentiate the fungi on the basis of their physiology. Blochwitz (1913) found that species of *Aspergillus* could be separated into groups according to their production of oxalic acid and pigment, their growth in media of different reactions, and their reaction to light, moisture and temperature. He did not make specific distinctions, however, without the aid of morphological characters. Woeltje (1918), on the other hand, who worked with 18 species of *Penicillium*, considered that the physiological properties of these fungi were of greater importance than their morphology. In his opinion it is impossible to make a complete differentiation of a series of species on the basis of morphology alone.

In the course of the present study, the physiological reactions of a number of soil fungi were observed with a view to determining their taxonomic value. A brief discussion follows:

1. *Pigment production.* Variations in the pigments produced by fungi in the substratum are often confusing. The changes which occur, however, generally follow changes in the reaction or composition of the medium. As pointed out by Thom and Church (1926) color production is a useful accessory character, but too much importance should not be placed on it in separating species. It is inadvisable to separate fungi which are morphologically alike because they may produce different colors in the medium.

2. *Gelatin liquefaction.* As mentioned above, gelatin liquefaction was helpful in separating certain of the *Penicillia*, but it was not of particular value in studying the other groups of fungi.

3. *Slime production.* Although the production of slime or mucous by fungi is used as a means of separating several genera, the conditions which influence its production have been studied very little. Four soil genera are distinguished on this basis, *Acrostalagmus* from *Verticillium*, and *Penicillium* from *Gliocladium*. While the writer made no special study of slime production, it was observed that this property was governed to some extent by the age of the culture. Cultures which were identified as *Verticillium* when young were later placed as *Acrostalagmus* as the conidial heads became enclosed in slime. It is possible that the moisture content of the medium, the humidity of the atmosphere, and the incubation temperature, as well as the age of the culture, are factors which influence slime production.

4. *Utilization of various carbohydrates as sole sources of carbon.* The selective fermentation of various carbohydrates, particularly the sugars, has come to be one of the most important means of differentiating the bacteria into species. Such reactions, in fact, are practically the sole means of separating certain groups, the members of which are indistinguishable on morphological characters. Although the fungi are more complex morphologically than the bacteria, it was thought not improbable that certain organisms or groups of organisms might be specific for certain carbohydrates. In order to determine whether the ability of an organism to attack carbohydrates can be employed to separate it from closely allied forms, the species under observation were grown on Dox synthetic solution, Thom (1910), with the following compounds serving as sole sources of carbon: dextrose, levulose, galactose, lactose, maltose, mannitol, lactic acid, oxalic acid, citric acid, malic acid, benzoic acid, and tartaric acid. The results are presented in table I.

From an examination of the data in this table it will be noted that, in general, the compounds which were readily available to one group of organisms were as easily attacked by many other groups which may or may not be alike morphologically. Dextrose, levulose and maltose served as excellent sources of carbon for most of the organisms tested; lactose, lactic acid, galactose, malic acid, tartaric acid and mannitol produced excellent growth with some species, while others failed to germinate, or grew poorly; benzoic acid in low concentration produced poor growth in a few cases, but, like oxalic acid, it was generally not attacked. Citric acid was utilized by *Aspergillus niger*, by *A. terreus*, and by one strain of *Trichoderma lignorum*.

#### NOMENCLATURE

Generic characters among most of the genera of the *Fungi Imperfecti* are not clearly defined, or perhaps it should be said that the individual fungi occurring in nature do not always fall within the boundary lines which have been established to delimit certain genera. While most of the genera occurring commonly in the soil have typical species which would not be confused with those of another group, there are many intermediate forms which overlap generic boundary lines and thereby tend to eliminate sharp distinctions between the groups.

Such variations are illustrated by the genus *Penicillium*, which is defined by Lindau (1910) as including those species that fruit asexually, with conidiophores branched to form a penicillate head, the conidiophores not

TABLE I. GROWTH OF FUNGI ON DOX SOLUTION WITH VARIOUS SOURCES OF CARBON.

Organism	Dex- trose 1 pct.	Levu- lose 1 pct.	Ga- lactose 1 pct.	Lactose 1 pct.	Maltose 1 pct.	Mannitol 1 pct.	Lactic acid 0.1 pct.	Oxalic acid 0.1 pct.	Citric acid 0.5 pct.	Malic acid 0.5 pct.	Benzoic acid 0.05 pct.	Tartaric acid 0.5 pct.
<i>Aspergillus niger</i> .....	xxx	xxx	x	G	xxx	G	x	O	xxx	xx	O	xxx
<i>Aspergillus Wentii</i> (soil) .....	xxx	xxx	x	G	xxx	G	x	O	xx	xx	O	xx
<i>Aspergillus Wentii</i> (USDA) * .....	xxx	xxx	xx	O	xxx	G	xxx	G	xx	xxx	O	xxx
<i>Aspergillus flavus</i> (soil) .....	xxx	xxx	xx	G	xxx	xxx	xxx	O	O	G	O	xx
<i>Aspergillus flavus</i> (USDA) .....	xxx	xxx	xxx	G	xxx	xxx	xxx	O	G	G	O	xxx
<i>Aspergillus terreus</i> .....	xxx	xxx	xx	xx	xxx	G	xxx	G	xx	xxx	G	x
<i>Aspergillus flavipes</i> .....	xxx	xxx	xxx	xxx	xxx	xx	G	O	O	xxx	x	x
<i>Aspergillus minutus</i> .....	xxx	xxx	xxx	xxx	xxx	x	O	O	O	O	O	O
<i>Aspergillus humus</i> .....	xxx	xxx	G	O	O	O	O	O	O	O	O	O
<i>Aspergillus glaucus</i> .....	xxx	xxx	xxx	xxx	xxx	x	O	O	O	O	O	O
<i>Penicillium purpurogenum</i> .....	xxx	xxx	xxx	G	xxx	G	xxx	O	O	xxx	O	O
<i>Penicillium roqueforti</i> .....	xxx	xxx	x	x	xxx	x	xxx	O	O	xxx	O	G
<i>Penicillium italicum</i> (soil) .....	xxx	xxx	xx	x	xxx	xxx	xxx	G	G	xxx	x	O
<i>Penicillium italicum</i> (USDA) .....	xxx	xxx	xxx	G	xxx	xxx	O	O	O	G	G	O
<i>Penicillium pinophilum</i> .....	xxx	xxx	xxx	O	xxx	O	xx	O	x	x	x	O
<i>Spicaria violacea</i> .....	xxx	xxx	xxx	x	xxx	xxx	O	O	O	O	x	O
<i>Trichoderma koningi</i> .....	xxx	x	xxx	G	x	x	O	O	O	x	O	O
<i>Trichoderma lignorum</i> .....	xxx	xxx	xxx	xxx	x	xxx	x	O	G	x	x	O
<i>Trichoderma lignorum</i> No. 198 .....	xxx	xxx	xxx	xxx	x	xxx	xxx	O	xx	xx	x	O
<i>Trichoderma glaucum</i> .....	xxx	xxx	xxx	G	G	xxx	G	O	x	G	O	O

Germination only, G.

No growth, O.

Typical growth, fruiting, xxx.  
 Fair growth, not fruiting, xx.  
 Poor growth, x.

\* Species designated as USDA were subcultures of cultures received from Dr. Thom.

inflated at the apex, and the head not enclosed in slime. Species producing typical penicillate fructifications are recognized readily, but it is those forms which deviate from the type that present difficulties. Considerable uncertainty often arises in differentiating between *Penicillium* and *Spicaria*, a closely related genus, which is defined by Lindau (1910) as including species with the conidiophores branched, usually in whorls, but also alternate or opposite, with the conidia borne in chains on terminal sterigmata, and not forming penicillate heads. This genus likewise is represented by typical species, yet in considering many of the fungi as they are found in nature, it is often difficult to draw a sharp line of distinction between *Penicillium* and *Spicaria*. This difficulty was experienced frequently in examining organisms isolated from the soil.

In distinguishing between these two genera it will be found that the conidiiferous cells of *Penicillium* are grouped more nearly parallel in the head than those of *Spicaria*, the latter arising at a considerably greater angle. The conidial head in *Penicillium*, therefore, is generally quite compact in comparison with the loose, straggling groups of chains in *Spicaria*. A further distinction is that there are seldom more than five or six conidial chains produced from a single conidiophore in *Spicaria*, while the heads of *Penicillium* are composed of many chains. It is not uncommon, however, to find in a culture of a typical *Penicillium* short stalks arising from aerial mycelium which bear but one or two conidial chains.

The genus *Gliocladium* differs from *Penicillium* in having the penicillate head enclosed in a sheath of slime or mucous. In the cultures of the single species of the genus isolated in this study, the production of slime was marked, leaving no doubt as to the identity of the genus.

Identification of members of the genus *Aspergillus* is a much simpler matter so far as determining the genus is concerned. In this genus are included those species which continue to reproduce asexually with conidiophores inflated at the apex to form a vesicle, which bears conidia in chains on branched or unbranched sterigmata. The writer is not inclined to recognize either the genus *Sterigmatocystis*, which was erected by Cramer to include forms having the sterigmata in two series, or the genus *Citromyces* Wehmer, the species of which have the power to produce citric acid. Physiological properties are not sufficiently specific among the fungi to be used as generic characters, and it is doubtful if even species can be separated on such characters alone. Neither do members of the genus having double sterigmata show sufficient differences from those with single sterigmata to warrant placing them in a separate genus. Branching of sterigmata should be used to distinguish species rather than genera.

No attempt has been made in this paper to identify members of the genus *Aspergillus* which produce perfect stages as species of *Eurotium*. Since the asexual stage is the one commonly observed, while the sexual fruiting structures are often transient in their appearance, it seemed desirable to consider only the conidial type of fructification as being of primary importance. Sclerotia production is common in a number of soil species of this genus. The production of perithecia was observed only in *Aspergillus glaucus*.

Another genus of considerable importance in the soil is *Trichoderma*. Difficulty is sometimes experienced in distinguishing members of this genus from those of *Acrostalagmus*. The conidiophores of *Trichoderma* are



branched alternately or oppositely, while those of a typical *Acrostalagmus* should be in whorls or verticils of the *Verticillium* type. Some cultures of *Trichoderma lignorum*, which were isolated from the soil, often showed branching closely approaching the *Acrostalagmus* type, while the conidiphores of a culture of *A. cinnabarinus* obtained from another source failed to show the typical verticillate branching.

The generic characters of *Acrostalagmus* are evidently not sharply defined. Young cultures which show the verticillate branching might be classed as *Verticillium* before the heads become enclosed in slime, while if the typical whorls are absent, they might easily be placed as *Trichoderma*.

### DESCRIPTION OF SPECIES

In addition to such standard mycological references as Engler and Prantl (1900), Rabenhorst (1910) and Saccardo (1882), many sources of information were consulted in identifying the species of fungi isolated in this work. Thom's key (1910) is a valuable aid in determining species of *Penicillium*. The key presented by Biourge (1923) in his recent monograph of the genus *Penicillium* is rather complex and difficult to follow. Biourge has attempted to revive the specific name *Penicillium glaucum*, the desirability of which is very questionable. Thom (1910) has shown that this term has been applied loosely to a collection of green forms, and that there is no evidence to indicate what species Link had under observation when the name was first used. It seems advisable, therefore, that the term should be discarded.

For the genus *Aspergillus* the classification presented by Thom and Church (1926) was found most satisfactory. The taxonomic literature relating to the less common genera is limited and identification of species with any degree of certainty is difficult, based as it must be solely upon the meager cultural data available. The published descriptions in many cases not only fail to include the essential details of morphology, but information as to the culture medium, temperature, and incubation period is entirely lacking.

Decision to create the new species described here was reached only after comparison with authentic cultures, when available, and careful examination of the literature failed to reveal sufficient similarities with the organism concerned to justify placing it in any of the established species. That new species of fungi would be isolated from the soil is not to be unexpected in view of the fact that few systematic studies of the fungous flora of agricultural soils have been made.

### ASPERGILLUS Michell

The genus *Aspergillus* comprises one of the most numerous groups of fungi found in the soil. The fact that species of this genus have been isolated by practically every investigator who has studied the soil fungous flora indicates that they are widely distributed in soils regardless of location or cropping history. They were found to be regularly present in all of the soils examined by the writer. The following species were isolated:

1. *Aspergillus minutus*, sp. nov. (fig. 1). One of the very common groups of organisms found in the soils studied is a series of forms which apparently belong to the *Aspergillopsis* group of Sopp. (1912); and which

were described by him as a species of *Penicillium*. They are closely related to such forms as *Penicillium hypo-janthinum* and *P. roseo-cinnabarinum* of Biourge (1923). Placing such forms in the genus *Penicillium* does not seem to be justified, however, when certain characters which closely ally them with *Aspergillus* are considered. The apical ends of the conidiophores of these organisms enlarge to form a vesicle, which, though small, is nevertheless distinct. The sterigmata, which occur in two series, are likewise characteristic of *Aspergillus* and do not resemble the conidiiferous cells of *Penicillium*. The writer is therefore inclined to consider descriptions placing such forms in the genus *Penicillium* as incorrect.

Since no suitable name for the organism was found in the literature, the species name *Aspergillus minutus* was applied. The following description is based on cultural examinations of a large number of isolations and is made broad enough to include a group of closely related strains.

Colonies on Czapek's agar white at first, becoming dark olive gray (LI)\* in seven to ten days with the appearance of fruiting areas, turning dark gray to brown in two weeks, and becoming various shades of brown or almost black with age. Surface cottony. Reverse shades of yellow and orange, the color often deepening to brown with age. Conidiophores septate, arising as short side branches of aerial mycelium, erect, 30 to 60 $\mu$  in length by 3 $\mu$  in diameter; rarely attain height of 125 $\mu$ . Heads usually round and radiate in young cultures, later tending toward calyptriform.

Fig. 1. *Aspergillus minutus*, sp. nov.  
a, c, conidiophores with round, young heads; b, older, calyptriform head (x 25); d, fruiting structure, showing globose vesicles and double sterigmata (x 250); conidia (x 300).

Vesicles small, 8.0 to 18.0 $\mu$  in diameter, globose, smooth; sterigmata in two series, primary 4.8 to 6.5 $\mu$  by 3.5 to 3.8 $\mu$ , secondary 4.8 by 3.2 $\mu$ . Conidia globose, verrucose, light brown in mass, 3.2 to 4.5 $\mu$  in diameter, mode 3.5 $\mu$ . Sclerotia or perithecia not observed.

Habitat: Soil.

2. *Aspergillus humus*, sp. nov. (fig. 2). Sopp's (1912) *Aspergilloides* section of *Penicillium* includes a group of fungi in which the slightly inflated apex of the main conidiophore bears several secondary branches, which he termed sterigmata. These secondary branches also enlarge at the apex and bear conidial chains on sterigmata. Such species may correctly be considered as being closely related to both *Aspergillus* and *Penicillium*, and unless a separate genus is created for them, their proper taxonomic position presents a problem.

The writer does not agree with Sopp in his inclusion of such organisms in the genus *Penicillium*. This opinion is based on an examination of his

\* Roman numerals refer to plate numbers in Ridgway's color chart (1912).

drawings and descriptions, since cultures of his species were not available, and on a study of similar organisms which were isolated from the soil. The sterigmata of Sopp's species, as shown by his drawings, are borne on the apex of the inflated conidiophore in a manner typical of many species of *Aspergillus*, while the penicillate type of fructification is lacking. As stated by Sopp, they bear some morphological resemblance to *Citromyces* and might possibly be so classified if they have the power of producing citric acid. Sopp did not include data on citric acid production. The writer, however, prefers to include the *Citromyces* section in the genus *Aspergillus*, rather than in *Penicillium*.

If all, or even a majority of the conidiophores in a culture of this group of organisms showed the type of prolific branching described, and shown in fig. 2a, it would probably be desirable to create a new genus for such forms, rather than to place them in a genus of which they are not typical, such as *Aspergillus*. In the cultures isolated from the soil by the writer, however, the branched conidiophores are not predominant, the most common type being the unbranched conidiophores which arise directly from the substratum and bear their conidia in chains on unbranched sterigmata (fig. 2d). The sterigmata are usually somewhat scattered over the surface of the vesicle, which gives the head a loose, straggling appearance in contrast to the compact heads common to most species of *Aspergillus*. This straggling appearance is particularly characteristic of the heads borne on the many-branched conidiophores.

Since the penicillate type of fructification is lacking, while many of the characteristics of *Aspergillus* are present, it seems that fungi of this group belong either in the genus *Aspergillus* or in a newly created genus. Revising the generic description of *Aspergillus* to include forms with branched conidiophores (and showing the other generic characters) would permit the inclusion of this group without difficulty. The writer has chosen to place these fungi in *Aspergillus*, provisionally at least, rather than to erect a new genus. Since the members of this group which were isolated from the soil differed in cultural details from Sopp's species, his specific names were not applicable, and the soil strains were classified as a new species of *Aspergillus*, *A. humus*. The cultural characterization is as follows:

Colonies on Czapek's agar white at first, becoming calla green (V) or cedar green (VI) in five to seven

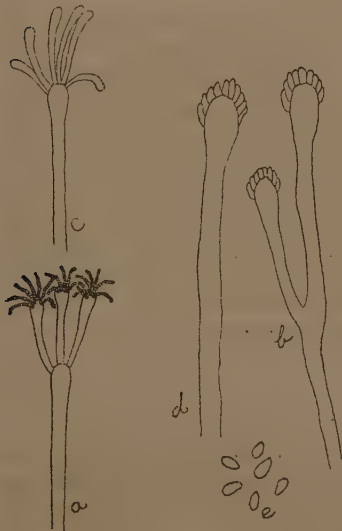


Fig. 2. *Aspergillus humus*, sp. nov. a, conidiophore with fertile branches arising apically, showing straggling type of head; c, conidiophore with sterile branches (xabout 150); b, conidiophore with lateral branch; d, unbranched conidiophore and single sterigmata; e, conidia (xabout 200).

days, changing thru brownish olive shades with age (XXX), and finally becoming snuff brown (XXIX) in six to seven weeks. Reverse of colony various shades of greenish brown. Surface powdery, consisting of dark, multiseptate mycelium. Conidiophores arise directly from the substratum or as branches of mycelium close to the substratum, multiseptate, variable in length, 200 to 700 $\mu$ ; pitted; three to ten or twelve secondary branches often arise apically from the main conidiophore, 35 to 125 $\mu$  in length, fertile (fig. 2a) or sterile (fig. 2c). Lateral branching (fig. 2b) is also common. Both branched and unbranched conidiophores are present in the same culture. Heads vary from loose aggregates of chains to fairly compact, calyptriform masses. Vesicles smooth, elongate, not sharply differentiated from the conidiophore, 18 to 30 $\mu$  by 12 to 20 $\mu$ . Sterigmata always in one series, oblong to lageniform, 8.0 to 15.0 $\mu$  long by 4.0 to 5.0 $\mu$  broad. Conidia ovoid to elliptical, smooth, 5.0 to 8.0 $\mu$  by 4.0 to 6.5 $\mu$ . Sclerotia or perithecia not observed. Does not grow on gelatin.

Habitat: Soil.

3. *Aspergillus fumigatus* Fresenius. This species was isolated in larger numbers from the soil than any other single organism.

4. *Aspergillus niger* van Tieghem. *A. niger* is widely distributed in soils, but it is generally found less commonly than *A. fumigatus*.

5. *Aspergillus terreus* Thom and Church. This species ranked second to *A. fumigatus* in frequency of occurrence.

6. *Aspergillus Wentii* Wehmer. A series of fungi belonging to this species was isolated, at one end of which is a form that is never floccose, and at the other end one showing the deep floccosity of the type species. Several intermediate strains show varying degrees of floccosity. This characteristic remained constant thru a long period of culturing. When young the floccose soil strains are indistinguishable from the type, but with age the type species becomes old gold (XVI) to medal bronze (IV) in color, while the soil strains remain a light honey yellow (XXX).

7. *Aspergillus flavipes* Bainier. *A. flavipes* has not been reported previously from the soil, altho Thom and Church (1926) mention having received cultures of it from Waksman's soil studies. It is one of the less common soil *Aspergilli*.

8. *Aspergillus flavus* Link.

9. *Aspergillus versicolor* (Vuillemin) Tiraboschi.

10. *Aspergillus clavatus* Desmasieres.

11. *Aspergillus glaucus* Link.

12. *Aspergillus koningi* Oudemans.

13. *Aspergillus candidus* Link.

The last named species were isolated only infrequently, *A. koningi* but once. *A. koningi* is not listed by Thom and Church (1926) with their accepted species. The soil fungus was so classified, however, since it corresponded more closely with the original description of *A. koningi* (1902) than of any other species.

Four unidentified species of *Aspergillus* were isolated.

#### PENICILLIUM Link

1. *Penicillium expansum* Link emended Thom. Waksman's statement (1916) that *Penicillium* is one of the most common soil genera is



borne out by the present work. Together with *Aspergillus* and *Trichoderma*, it ranks as one of the most important soil groups. Species of the genus were found to be constantly present in all of the soils examined. The following species were isolated:

1. *Penicillium expansum* Link emended Thom. This is the most common soil *Penicillium*. It also ranks as one of the most common of all soil fungi.

2. *Penicillium luteum* Zukal. The frequent occurrence of this organism in soils and its ability to oxidize elemental sulfur to the sulfate form were reported in a previous paper (1923). This is recognized as a group species which includes those *Penicillia* producing yellowish or reddish sclerotia. Both the red and yellow forms were isolated. Sopp (1912) believed that the species he described as *P. sulfureum* and *P. sanguineum* might also be classed as *P. luteum*.

When carried in culture for long periods, the cultures isolated from the soil usually lost their characteristic property of producing yellow sclerotia, which is the distinguishing character when first isolated, and changed over to a pale green conidial stage.

Further evidence of the ability of this fungus to oxidize sulfur, both in the soil and in solution cultures, is reported by Bollen (1925), who found the yellow forms of this species occurring in large numbers in sulfur-treated soils.

3. *Penicillium pinophilum* Hedgecock.

4. *Penicillium chrysogenum* Thom.

5. *Penicillium roqueforti* Thom.

6. *Penicillium lilacinum* Thom. Several isolations were made of an organism having the general appearance and characteristics of *P. lilacinum* Thom, but showing certain variations from the type species, a culture of which was received from Dr. Thom. When grown on Czapek's agar, the conidiophores of the soil strain reach a height of 300 to 700 $\mu$  as opposed to 100 $\mu$  for the type species. The surface of the soil culture has a powdery appearance with little indication of the floccosity of the type. The two organisms showed no differences in their reaction to gelatin.

7. *Penicillium funiculosum* Thom.

8. *Penicillium purpurogenum* Stoll.

9. *Penicillium commune* Thom.

10. *Penicillium rugulosum* Thom.

11. *Penicillium duclauxi* Delacroix.

12. *Penicillium italicum* Wehmer.

The most common soil members of this genus are *P. expansum*, *P. chrysogenum*, and *P. pinophilum*.

#### GLIOCLADIUM Corda

The production of a slime or mucous which surrounds the penicillate fruiting head is the only character which separates the genus *Gliocladium* from the genus *Penicillium*. One species of this genus, which was isolated frequently from the soils studied, was identified as *Gliocladium penicilloides* Corda. While positive identification as the original organism with which

Corda worked is impossible from the data given in his description, the soil strain was placed as such as an alternative to describing a new species. The cultural characterization is as follows:

Colonies on Czapek's agar pure white, broadly spreading, sometimes fuscus, with pale flesh-colored fruiting areas appearing in the center of the colony in seven to ten days. The fruiting heads are embedded in a slime, which gives the colony a moist appearance, resembling bacterial contamination. Reverse of colony colorless. Conidiophores septate, arising as branches of aerial mycelium, 50 to  $100\mu$  in height by  $3\mu$  in diameter. Fruiting heads typically penicillate, primary branches 15 to  $25\mu$  long by  $3.2\mu$  in diameter, secondary, 10 to 15 by  $2.5\mu$ , tertiary 10 to 14 by  $1.5\mu$ . Conidia oblong or bacillate, smooth, colorless,  $3.5$  to  $4.0\mu$  long by  $2.0\mu$  broad.

#### SPICARIA Harting

Altho the genus *Spicaria* is not one of the predominating soil groups, two species were isolated quite frequently. One of these was identified as *S. simplicissima* Oud. and the other is described as a new species, *S. violacea*.

*Spicaria violacea*, sp. nov. (fig. 3). This fungus can be recognized readily by the beautiful lavender to violet color of the colony. The cultural characterization is as follows:

Colonies on Czapek's agar white at first, becoming pale vinaceous lilac to vinaceous lavender (XLIV) in fourteen days, and deepening to dark lavender to slate violet (XLIV) with age. Reverse colorless. Surface cottony or slightly floccose, with broad, pure white margins. Surface growth consists of a dense network of thread-like, interlacing hyphae. Conidiophores short, up to  $100\mu$  in length, arising as branches of aerial mycelium, sometimes having single branches (fig. 3a), or two branches occurring oppositely, and bearing at the apex one to six (rarely more) long chains of conidia on sterigmata. Sterigmata club shaped,  $6.5\mu$  long by  $2.0\mu$  broad. Conidial chains long, up to 600 or  $700\mu$  in length. Conidia ellipitcal, smooth, hyaline,  $3.0$  to  $3.5\mu$  long by  $2.0$  to  $2.5\mu$  in width.

Habitat: Soil.

#### TRICHODERMA Persoon

Members of this genus are widely distributed in soils. Cultural studies were made on seven fungi belonging to the genus which were isolated at various times. These organisms constitute a series of intergrading forms, which show sufficient cultural differences to be considered as separate species when observed individually, but when the entire series is studied in parallel culture, the apparently sharp lines of difference disappear and natural groupings become evident. Four fairly well defined groups are recognized, the floccose green forms being classified as *T. kon-*

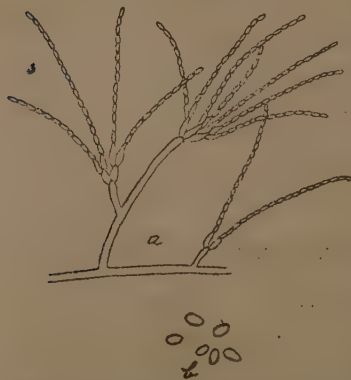


Fig. 3. *Spicaria violacea*, sp. nov. a, fruiting structure showing branched conidiophore and loose heads (xabout 200); b, conidia, (400).



Fig. 4. *Trichoderma glaucum*, sp. nov. a, b, c, showing conidiophores arising alternately, oppositely, or irregularly (xabout 250); d, swollen and distorted hyphae and conidiophores, which occur commonly in culture; (xabout 250); e, conidia, (x300).

*ingi* and the tufted green strains, which are not floccose, as *T. lignorum*. Members of a third group, which produce a restricted, yellow or greenish-yellow colony, are described in this paper as a new species, *T. glaucum*. A fourth group of white to gray forms, which were never definitely identified, probably deserves separate specific rank. Comparative morphological and cultural characters of the soil cultures are given in table II. The following descriptions are based on cultures isolated from the soil:

1. *Trichoderma glaucum*, sp. nov. (fig. 4). Colonies on Czapek's agar spreading; at first only a thin, sterile, mycelial film covers the surface of the medium. White aerial mycelium develops in five to seven days, followed in ten days by the appearance of the yellow fruiting areas, which change thru shades of chartreuse yellow to citron or lime green (XXXI). Vegetative mycelium hyaline, 3.0 to 6.0 $\mu$  thick, multiseptate, and freely branched; cells are often

short and swollen or barrel shaped (fig. 4d). Conidiophores arise as side branches, alternately, oppositely or irregularly branched (fig. 4a, b, c); up to 60 $\mu$  in height by 3.0 $\mu$  in width. Conidial heads 6.5 $\mu$  to 10.0 $\mu$  in diameter. Conidia smooth, hyaline, ovoid, 3.8 to 5.0 $\mu$  by 2.5 to 3.0 $\mu$ , mode 4.0 by 3.0 $\mu$ .

TABLE II. COMPARATIVE CULTURAL DATA OF SPECIES OF  
*TRICHODERMA*

Medium	<i>T. lignorum</i>	<i>T. lignorum</i> No. 198	<i>T. koningi</i>	<i>T. glaucum</i>
Czapek's agar surface	Restricted, tufted; gnaphalium green to slate olive green (olive greens)	Restricted; tufted dark American green (deep greens)	Floccose; glaucous green to asphodel green	Felt, spreading; chartreuse yellow to yellow green or lime green
Conidiophores	Alternate or opposite 70 x 3.0 u	Whorled 70 x 3.0 u	Alternate or opposite 25 x 3.0 u	Alternate or opposite 35 x 3.0 u
Conidia	Globose to ovoid 2.8 to 3.2 u	Globose to ovoid 2.8 to 3.2 u	Elliptical to oblong 3.2 to 4.8 by 1.8 to 3.0 u	Globose to ovoid 3.5 to 4.0 by 2.8 to 3.2 u
Bean Agar	Subfloccose, tufted yellow to olive green	Subfloccose, tufted leaf green	Very floccose; color same as on Czapek's	Scant, transparent growth; not fruiting
Gelatin	Rapid liquefaction; straw color	Rapid liquefaction amber color	Rapid liquefaction; no color.	Rapid liquefaction no color
Milk	Poor growth; digestion complete; dark amber color	Poor growth; digestion less rapid than type; amber color	Good growth; digestion less rapid than <i>lignorum</i> ; light amber color	Poor growth; digestion complete; dark amber color
Rice	Typical growth	Typical growth	Typical growth	Cottony, light green

Habitat: Soil.

2. *Trichoderma koningi* Oudemans. Colonies on Czapek's agar spreading, floccose, white at first, but becoming green in four to five days. Changes thru a series of greens (XLI) in ten to fourteen days, and remains this color in old cultures. Reverse of colony colorless. Vegetative hyphae septate, hyaline. Conidiophores arise as branches of aerial mycelium, alternate or opposite, up to  $25\mu$  in height by  $3.0\mu$  in diameter, di- or trichotomously branched. Fruiting heads  $6.5$  to  $10.0\mu$  in diameter; conidia oblong to elliptical,  $3.2$  to  $4.8\mu$  in length by  $1.8$  to  $3.0\mu$  in width, smooth, hyaline.

3. *Trichoderma lignorum* (Tode) Harz. Colonies on Czapek's agar consist of broadly spreading hyaline mycelium; fruiting areas appear as cottony tufts, white at first, becoming gnaphalium green (XLVII) in four to five days. Color does not change in old cultures. Reverse colorless. Conidiophores septate, arising as branches of aerial mycelium, up to  $70\mu$  in height by  $3.0\mu$  in diameter, di- or trichotomously branched, occasionally forming whorls. Conidia heads  $6.5$  to  $10.0\mu$  in diameter. Conidia smooth, globose to ovoid,  $2.8$  to  $3.2\mu$  in diameter.

Habitat: Soil.

#### MISCELLANEOUS SPECIES

Representatives of numerous other genera occur more or less commonly in the soil in addition to those which have been considered in some detail. Among the most important of these are *Alternaria*, *Cladosporium*, *Mucor* and *Rhizopus*. *Alternaria humicola*, *Cladosporium herbarum*, *Rhizopus nigricans*, and various species of *Mucor* are common in cultivated soils. Species of *Cephalosporium*, *Fusarium*, *Monilia*, *Verticillium* and *Isaria* were isolated, but specific determination was not completed.

The following species were isolated: *Alternaria humicola* Oudemans, *Cladosporium herbarum* Persoon, *Acrostalagmus albus* Preus, *Verticillium terrestris* (Link) Lindau, *Chaetomella horrida* Oudemans, *Stachybotrys lobulata* Berk., *Mucor glomerula* Lendner, *M. geophilus* Oudemans, *M. racemosus* Fresenius, *Rhizopus nigricans* Ehrenberg, and *Zygorrhynchus vuilleminii* Namyslowski.

A complete list of the fungi isolated in the present study is given in table III.

#### SUMMARY

Eleven soil plots under a definite system of cropping and fertilizer treatment were examined for their fungous flora, during the course of which study 44 species belonging to 14 genera were isolated and identified, in addition to unidentified species of four other genera.

Difficulties in the identification of soil fungi are discussed, among which are the lack of authentic cultures for comparison, and of adequate cultural descriptions.

Detailed morphological and physiological studies of a number of soil fungi are reported, including descriptions of four new species. These studies confirm the findings of previous investigators that there is a definite soil fungous flora, consisting principally of species of *Penicillium*, *Aspergillus*, *Trichoderma*, *Cladosporium*, *Rhizopus* and *Mucor*, with representatives of other genera occurring with varying regularity.



TABLE III. LIST OF FUNGI ISOLATED FROM THE SOIL.

1. *Penicillium expansum* Link em. Thom
2. " *pinophilum* Hedgcock
3. " *chrysogenum* Thom
4. " *luteum* Zukal
5. " *italicum* Wehmer
6. " *roqueforti* Thom
7. " *lilacinum* Thom
8. " *purpurogenum* Stoll
9. " *funiculosum* Thom
10. " *commune* Thom
11. " *intricatum* Thom
12. " *duclauxi* Delacroix
13. " *rugulosum* Thom
14. *Aspergillus fumigatus* Fres.
15. " *terreus* Thom and Church
16. " *niger* van Tieghem
17. " *flavus* Link
18. " *nidulans* Eidam
19. " *glaucus* Link
20. " *clavatus* Desmasieres
21. " *Wentii* Wehmer
22. " *flavipes* Bainier
23. " *versicolor* (vuillemin) Tiraboschi
24. " *candidus* Link
25. " *koningi*, Oud.
26. " *minutus*, sp. nov.
27. " *humus*, sp. nov.
28. *Trichoderma koningi* Oud.
29. " *lignorum* (Tode) Harz
30. " *glaucum*, sp. nov.
31. *Gliocladium penicilloides* Corda
32. *Spicaria simplicissima* Oud.
33. " *violacea*, sp. nov.
34. *Verticillium terrestre* (Link) Lindau
35. *Acrostalagmus albus* Preuss
36. *Alternaria humicola* Oud.
37. *Cladosporium herbarum* Pers.
38. *Stachybotrys lobulata* Berk.
39. *Chaetomella horrida* Oud.
40. *Rhizopus nigricans* Ehrenb.
41. *Mucor glomerula* Lend.
42. " *geophilus* Oud.
43. " *racemosus* Fres.
44. *Zygorrhynchus vuilleminii* Namys.
45. *Cephalosporium* sp.
46. *Fusarium* sp.
47. *Monilia* sp.
48. *Isaria* sp.

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## APPENDIX

The following list of fungi which are reported in the literature as having been isolated from the soil is believed to be complete. Some of the species listed by Jensen (1912) as having been isolated from the soil by European workers have been omitted where it is definitely stated that they were isolated from objects in contact with the soil and not from the soil itself. Such organisms cannot properly be considered as true soil forms. It is possible, however, that some of those included may have come from similar sources, since much of the information in the literature is indefinite in this respect. The species reported by Sopp (1912) as having been isolated from the soil were not included in the list, since it is difficult to tell from his data whether they were actually obtained from the soil or from organic matter in contact with the soil.

In preparing the list of fungi no attempt was made to eliminate synonymy, which is probably responsible for some duplication. The names used by the different investigators were accepted in each case.

## PHYCOMYCETES

Order *Mucorales*Family *Mucoraceae*

Species	Literature reference
1. <i>Absidia orchidis</i> (Vuill) Hagem	Hagem (1907); Waksman (1917); Dale (1914).
2. " <i>glaucia</i> Hagem	Hagem (1907); Lendner (1908); Dale (1914); Pratt (1918).
3. " <i>Lichtheimi</i> Lendner	Lendner (1908); Waksman (1917).
4. " <i>caerulea</i> Bainier	Oudemans and Koning (1902).
5. " <i>spinosa</i> Lendner	Lendner (1908).
6. " <i>cylindrospora</i> Hagem	Waksman (1917); Pratt (1918).
7. <i>Rhizopus nigricans</i> Ehrenb.	Adametz (1886); Hagem (1907); Jensen (1912); McLean and Wilson (1914); Waksman (1917); Pratt (1918); Werkenthin (1916); Takahashi (1919); Rathbun (1918); Abbott (1923).
8. " <i>arrhizus</i> Fischer	Dale (1914).
9. " <i>nodosus</i> Namys	Lendner (1908); Hagem (1907); Waksman (1917).
10. <i>Mucor hiemalis</i> Wehmer	Waksman (1916), (1917); Hagem (1907); Lendner (1908); Jensen (1912).
11. " <i>microsporus</i> Namys	Waksman (1917); Namyslawski (1910).
12. " <i>circinelloides</i> van Tiegh	Waksman (1917); Dale (1914); Jensen,

- (1912); Takahashi (1919); Pratt (1918).  
 13. " *plumbeus* Bonorden Waksman (1917); Jensen (1912); Dale (1914); Pratt (1918).  
 14. " *racemosus* Fres. Lendner (1908); Hagem (1907); Adametz (1886); Jensen (1912); Waksman (1917); Takahashi (1919); McLean and Wilson (1914); Werkenthin (1916); author.  
 15. " *silvaticus* Hagem Hagem (1907); Waksman (1917).  
 16. " *botryoides* Lendner Lendner (1908); Jensen (1912); Waksman (1917); Rathbun (1918).  
 17. " *saturninus* Hagem Hagem (1910); Waksman (1917).  
 18. " *sphaerosporus* Hagem Hagem (1907); Dale (1914); Waksman (1917); Pratt (1918).  
 19. " *glomerula* (Bainier) Lendner Lendner (1908); Dale (1914); Waksman (1917); Abbott (1923).  
 20. " *flavus* Bainier Lendner (1908); Hagem (1907); Waksman (1917).  
 21. " *Ramannianus* Moeller Hagem (1907); Dale (1914).  
 22. " *adventitus* Oud. Oudemans (1902); Takahashi (1919).  
 23. " *strictus* Hagem Hagem (1907).  
 24. " *mucedo* Brefeld Hagem (1907); Jensen (1912); Adametz (1886).  
 25. " *Christianensis* Hagem Hagem (1910).  
 26. " *dispersus* Hagem Hagem (1910).  
 27. " *genevensis* Lendner Lendner, see Jensen (1912); Hagem (1910).  
 28. " *lamprosporus* Lendner Lendner, see Jensen (1912).  
 29. " *griseo-cyanus* Hagem Hagem (1912); Lendner (1908).  
 30. " *corticulus* Hagem Hagem (1910).  
 31. " *Jansenni* Lendner Lendner (1908); Pratt (1918).  
 32. " *dimorphosporus* Lendner Lendner (1908).  
 33. " *geophilus* Oud. Oudemans (1902); Abbott (1923).  
 34. " *spinescens* Lendner Pratt (1918).  
 35. " *ambiguus* Vuill. Goddard (1913).  
 36. " *stolonifer* Ehrenb. Goddard (1913); Adametz (1886).  
 37. " *heterogamus* Rathbun (1918).  
 38. *Zygorrhynchus Vuilleminii*, Namys Waksman (1917); Abbott (1923); Namyslowski, see Jensen (1912).  
 39. " *Moelleri* Vuill. Jensen (1912).

Family *Thamnidaceae*

40. *Thamnidium elegans* Link Jensen (1912).

Family *Pilobolaceae*

41. *Pilaira anomala* (Cesati) Schroeter Koning, see Jensen (1912).

Family *Mortierellaceae*

42. *Mortierella subtilissima* Oud. Koning (1902).  
 43. " *pusilla* Oud. Koning (1902).  
 44. " *isabellina* Oud. Koning (1902).  
 45. " *humicola* Oud. Koning (1902).

Family *Chaetocladiaceae*

46. *Cunninghamella elegans* Lendner Lendner (1908).

Family *Saprolegniaceae*

47. *Aphanomyces laevis* de Bary See Jensen (1912).  
 48. *Pythium de Baryanum* Hesse Butler (1907).  
 49. " *intermedium* de Bary Butler (1907).  
 50. " *vexans* de Bary Butler (1907).  
 51. " *proliferum* de Bary Butler (1907).  
 52. " *rostratum* Butler Butler (1907).  
 53. " *monospermum* Pringsch. Butler (1907).



## ASCOMYCETES

## Order Sphaeriales

## Family Chaetomiaceae

- |     |   |                   |
|-----|---|-------------------|
| 54. | <i>Chaetomium olivaceum</i> Cooke and Ellis | Jensen (1912).    |
| 55. | <i>Chaetomium cochliodes</i> Palliser       | Waksman (1917).   |
| 56. | " <i>globosum</i> Kunze                     | Waksman (1917).   |
| 57. | " <i>funiculum</i> (?)                      | Waksman (1917).   |
| 58. | " <i>crispatum</i> Fuckeli                  | Takahashi (1919). |

## Order Sordariaceae

- |     |                                     |                |
|-----|-------------------------------------|----------------|
| 59. | <i>Pleurage verruculosus</i> Jensen | Jensen (1912). |
| 60. | <i>Sporormia fasciculata</i> Jensen | Jensen (1912). |

## FUNGII IMPERFECTI

## Order Sphaeropsidales

## Family Sphaerioidaceae

- |     |                                    |                               |
|-----|------------------------------------|-------------------------------|
| 61. | <i>Chaetomella horrida</i> Oud.    | Koning (1902); Abbott (1923). |
| 62. | " <i>tortilis</i> Delacroix        | Koning, see Jensen (1912).    |
| 63. | <i>Sphaeronema Fagi</i> Oud.       | Koning (1902).                |
| 64. | <i>Coniothyrium Fuckelii</i> Sacc. | Waksman (1917).               |

## Order Moniliales

## Family Moniliaceae

- |     |  |  |
|-----|--|--|
| 65. | <i>Sachsia albicans</i> Bay            | Jensen (1912).   |
| 66. | <i>Oospora lactis</i> (Fres) Sacc.     | Adametz (1886).  |
| 67. | " <i>variabilis</i> (?)                | Dale (1914).   |
| 68. | <i>Monilia sitophila</i> (Mont) Sacc.  | Waksman (1917).  |
| 69. | " <i>candida</i> Bonord.               | Adametz (1886).  |
| 70. | " <i>humicola</i> Oud.                 | Koning (1902); Waksman (1917).   |
| 71. | " <i>fimicola</i> Cost. et Matr.       | See Jensen (1912).   |
| 72. | " <i>geophila</i> Oud.                 | Koning (1902).   |
| 73. | " <i>koningi</i> Oud.                  | Koning (1902); Dale (1914); Goddard (1913).  |
| 74. | <i>Oidium lactis</i> Fres.             | Waksman (1917); Adametz (1886).  |
| 75. | <i>Cephalosporium acremonium</i> Corda | Koning (1902); Dale (1914); Waksman (1917).  |
| 76. | " <i>koningi</i> Oud.                  | Jensen (1912); Koning (1902); Waksman (1917).  |
| 77. | " <i>curtipes</i> Sacc.                | Waksman (1917).  |
| 78. | " <i>humicola</i> Oud.                 | Koning (1902).   |
| 79. | <i>Trichoderma koningi</i> Oud.        | Oudemans and Koning (1902); Jensen (1912); Dale (1914); Abbott (1923); Takahashi (1919); Waksman (1917); Goddard (1913); Rathbun (1918). |
| 80. | " <i>lignorum</i> (Tode) Harz          | Jensen (1912); Waksman (1917); Dale (1914); author.  |
| 81. | " <i>album</i> Preuss.                 | Waksman (1917).  |
| 82. | " <i>glaucum</i> Abbott                | Author.  |
| 83. | " <i>nigrovirens</i> Goddard           | Goddard (1913).  |
| 84. | <i>Corethropsis paradoxa</i> Corda     | Jensen (1912).   |
| 85. | <i>Aspergillus niger</i> van Tiegh.    | Waksman (1917); Dale (1914); Rathbun (1918); Takahashi (1919); Abbott (1923).  |
| 86. | " <i>fumigatus</i> Fres.               | Waksman (1917); Jensen (1912); Abbott (1923); Takahashi (1919).  |
| 87. | " <i>nidulans</i> Eidam                | Waksman (1917); Goddard (1913); Takahashi (1919); Abbott (1923).   |
| 88. | " <i>diversicolor</i> Vuill.           | Waksman (1917).  |
| 89. | " <i>calypttratus</i> Oud.             | Waksman (1917); Oudemans and Koning (1902); Goddard (1911).  |
| 90. | " <i>fuscus</i> Schieman               | Waksman (1917).  |

91. " *flavus* Link Waksman (1917); Abbott (1923).  
 92. " *clavatus* Desmas. Waksman (1917); Abbott (1923).  
 93. " *repens* de Bary Waksman (1917); Dale (1914).  
 94. " *globosus* Jensen Jensen (1912); Waksman (1917); Dale (1914).  
 95. " *terricola* March Scales (1914).  
 var. *Americana*  
 96. *Aspergillus koningi* Oud. Oudemans and Koning (1902); Abbott (1923).  
 97. " *candidus* Pers. Dale (1914); author.  
 98. " *glaucus* Link Adametz (1886); Takahashi (1919); Goddard (1911); Abbott (1923).  
 99. " *venetus* Mass. Werkenthin (1916).  
 100. " *terreus* Thom and Church See Thom and Church (1926); author.  
 101. " *orysae* Ahlburg Takahashi (1919).  
 102. " *Wentii* Wehmer See (1926); author.  
 103. " *tamaris* Kita See (1926).  
 104. " *flavipes* Bainier See (1926); author.  
 105. " *versicolor* (Vuill) Author.  
 Tiraboschi  
 106. *Aspergillus minutus* Abbott Author.  
 107. " *humus* Abbott Author.  
 108. *Citromyces glaber* Wehmer Dale (1914).  
 109. *Penicillium commune* Thom Waksman (1917); Abbott (1923).  
 110. " *chrysogenum* Thom Jensen (1912); Waksman (1917); Abbott (1923).  
 111. " *decumbens* Thom Waksman (1917); Abbott (1923).  
 112. " *digitatum* Sacc. Waksman (1917).  
 113. " *expansum* Link emend. Waksman (1917); Jensen (1912); Adametz (1886); Dale (1914); Abbott (1923).  
 Thom  
 114. *Penicillium italicum* Wehmer Waksman (1917); author.  
 115. " *oxalicum* Thom Waksman (1917).  
 116. " *notatum* Westling Waksman (1917).  
 117. " *viridicatum* West. Waksman (1917); Dale (1914); Pratt (1918).  
 118. " *atramentosum* Thom Waksman (1917).  
 119. " *rugulosum* Thom Waksman (1917); Dale (1914); Abbott (1923).  
 120. " *cyclopium* West. Waksman (1917); Dale (1914).  
 121. " *lividum* West. Waksman (1917); Dale (1914).  
 122. " *glaber* Wehmer Waksman (1917).  
 123. " *pfefferianus* Wehmer Waksman (1917).  
 124. " *desiscens* Oud. Waksman (1917); Jensen (1912).  
 125. " *geophilum* Oud. Oudemans and Koning (1902).  
 126. " *silvaticum* Oud. Oudemans and Koning (1902); Jensen (1912).  
 127. " *terrestre* Jensen Jensen (1912); Dale (1914).  
 128. " *lilacinum* Thom Waksman (1917); Dale (1914); author.  
 129. " *humicola* Oud. Oudemans and Koning (1902); Jensen (1912); Pratt (1918); Goddard (1913); Takahashi (1919).  
 130. " *luteum* Zukal Waksman (1917); Werkenthin (1916); Abbott (1923).  
 131. " *pinophilum* Hedg. Waksman (1917); Abbott (1923).  
 132. " *purpurogenum* (Stoll) Waksman (1917); author.  
 Thom  
 133. *Penicillium roqueforti* Thom Waksman (1917); Abbott (1923).  
 134. " *intricatum* Thom Waksman (1917); Dale (1914); Abbott (1923).  
 135. " *frequentans* West. Waksman (1917).  
 136. " *glaucum* Link Goddard (1913); Adametz (1886).

137. " *bicolor* Fr. Goddard (1913).  
 138. " *candidum* Link Takahashi (1919); Goddard (1911).  
 139. " *roseum* Link Takahashi (1919).  
 140. " *duclauxi* Delacroix Takahashi (1919); author.  
 141. " *funiculosum* Thom Abbott (1923).  
 142. *Gliocladium penicilloides* Corda Dale (1914); author.  
 143. *Scopulariopsis brevicaulis* Sacc. Waksman (1917).  
 144. " *communis* (?) Dale (1914).  
 145. " *repens* (?) Dale (1914).  
 146. " *rufulus* (?) Dale (1914).  
 147. " *roseum* (?) Dale (1914).  
 148. *Ambylosporium echinulatum* Oud. Koning (1902).  
 149. *Monosporium silvaticum* Oud. Koning (1902).  
 150. " *acuminatum* var. *terrestre* Sacc. Saccardo, see Jensen (1912).  
 151. *Allescheriella nigra* (?) Takahashi (1919).  
 152. *Botrytis cinerea* Pers. Waksman (1917); Takahashi (1919);  
 Dale (1914); Lendner (1908).  
 153. " *terrestris* Jensen Jensen (1912).  
 154. *Verticillium chlamydosporium* Goddard Goddard (1913).  
 155. *Verticillium terrestre* (Link) Lindau Waksman (1917).  
 156. " *glaucum* Bonord. Waksman (1917).  
 157. *Acrostalagmus albus* Preuss. Jensen (1912).  
 var. *varius* Jensen Waksman (1917); Abbott (1923).  
 158. *Acrostalagmus albus* Preuss.  
 159. " *cinnabarinus* Koning (1902); Waksman (1917); Goddard (1913).  
 var. *nana* Oud. Koning (1902); Rathbun (1918).  
 160. *Spicaria silvatica* Oud. Koning (1902); Jensen (1912); author.  
 161. " *simplicissima* Oud. Koning (1902).  
 162. " *decumbens* Oud. Koning (1902).  
 163. " *violacea* Abbott Author.  
 164. *Cephalothecium roseum* Corda Waksman (1917).  
 165. *Papulaspora pannosa* (?) Waksman (1917).  
 166. *Sepedonium chrysospermum* Bull. Waksman (1917).  
 167. *Nematogonium humicola* Oud. Koning (1902); Dale (1914).  
 168. *Trichothecium roseum* Link Jensen (1912); Dale (1914).  
 169. *Mycogone nigra* Morgan Jensen (1912).  
 170. *Ramularia magnusiana* (Sacc) Lindau Werkenthin (1916).  
 171. *Myceliophthora sulphurea* God. Goddard (1913).  
 172. *Coccospora agricola* Goddard Goddard (1913).  
 173. *Pachybasium humatum* Bonord. Goddard (1913).
- Family *Dematiaceae*
174. *Torula lucifuga* Oud. Koning (1902).  
 175. *Basisporium gallarum* Molliard Waksman (1917).  
 176. *Cladosporium herbarum* Pers. Waksman (1917); Dale (1914); Werkenthin (1916); Abbott (1923).  
 177. " *epiphyllum* Pers. Waksman (1917); Dale (1914).  
 178. *Hormodendrum hordei* Bruhne Jensen (1912); Waksman (1917).  
 179. " *cladosporioides* (Fres.) Sacc. Jensen (1912); Goddard (1913).  
 180. *Hormodendrum pallidum* Oud. Koning (1902).  
 181. *Helminthosporium interseminatum* Berk. et Rav. Dale (1914).  
 182. *Helminthosporium subulatum* Noes. Takahashi (1919).  
 183. *Alternaria humicola* Oud. Koning (1902); Dale (1914); Waksman (1917); Abbott (1923).  
 184. " *fasciculata* Cooke and Ellis Jensen (1912).  
 185. *Alternaria tenuis* Nees. Dale (1914); Takahashi (1919).  
 186. *Macrosporium commune* Rabenh. Pratt (1918).

- |      |   |                   |
|------|---|-------------------|
| 187. | <i>Stemphyllium piriforme</i> Bonord.             | Pratt (1918).     |
| 188. | " <i>paxianum</i>                                 | Pratt (1918).     |
| 189. | " <i>veruculosum</i> Zimm.                        | Takahashi (1919). |
| 190. | " <i>botryosum</i> Wallr.                         | Dale (1914).      |
| 191. | <i>Synsporium biguttatum</i> Preuss.              | Dale (1914).      |
| 192. | <i>Stachybotrys cylindrospora</i> Jensen          | Jensen (1912).    |
| 193. | " <i>atra</i> Corda                               | Jensen (1912).    |
| 194. | " <i>lobulata</i> Berk.                           | Author.           |
| 195. | <i>Dematium pullulans</i> de Bary                 | Waksman (1917).   |
| 196. | <i>Dicoccum aspera</i> Corda                      | Waksman (1917).   |
| 197. | <i>Acremoniella fusca</i> var. <i>minor</i> Corda | Waksman (1917).   |
| 198. | <i>Trichocladium asperum</i> Harz                 | Waksman (1917).   |
| 199. | <i>Periconia byssoides</i> Pers.                  | Pratt (1918).     |

## Family Stilbaceae

- |      |  |                                 |
|------|--|---------------------------------|
| 200. | <i>Stysanus stemonites</i> (Pers.) Corda | Waksman (1916); Goddard (1913). |
| 201. | <i>Tilachlidium humicola</i> Oud.        | Koning (1902).                  |

## Family Tuberculariaceae

- |      |                                  |                              |
|------|----------------------------------|------------------------------|
| 202. | <i>Fusarium angustum</i> Sherb.  | Waksman (1917).              |
| 203. | " <i>bullatum</i> Sherb.         | Waksman (1917).              |
| 204. | " <i>solani</i> , Ap. et Wr.     | Waksman (1917); Dale (1914). |
| 205. | " <i>orthoceras</i> Ap. et Wr.   | Waksman (1917).              |
| 206. | " <i>oxysporum</i> Sherb.        | Waksman (1917).              |
| 207. | " <i>caudatum</i> Wr.            | Waksman (1917).              |
| 208. | " <i>rubiginosum</i> (?)         | Dale (1914).                 |
| 209. | " <i>lini</i> Bolley             | Waksman (1916).              |
| 210. | " <i>affini</i> F. and L.        | Pratt (1918).                |
| 211. | " <i>dimerium</i> Penz.          | Pratt (1918).                |
| 212. | " <i>lanceolatum</i> Pratt       | Pratt (1918).                |
| 213. | " <i>acuminatum</i> Ell. and Ev. | Pratt (1918).                |
| 214. | " <i>sanguineum</i> Sherb.       | Pratt (1918).                |
| 215. | " <i>elegantum</i> Pratt         | Pratt (1918).                |
| 216. | " <i>idahoanum</i> Pratt         | Pratt (1918).                |
| 217. | " <i>tricothecioides</i> Woll.   | Pratt (1918).                |
| 218. | " <i>culmorum</i> Sherb.         | Pratt (1918).                |
| 219. | " <i>discolor</i> Sherb.         | Pratt (1918).                |
| 220. | " <i>subpallidum</i> Sherb.      | Pratt (1918).                |
| 221. | " <i>aridum</i> Pratt            | Pratt (1918).                |
| 222. | " <i>nigrum</i> Pratt            | Pratt (1918).                |
| 223. | " <i>radicola</i> Wr.            | Pratt (1918).                |
| 224. | " <i>oxysporum</i> Schlecht.     | Werkenthin (1916).           |
| 225. | <i>Myrothecium roridum</i> Tode  | Waksman (1917).              |

## Mycelia Sterilia

- |      |                                 |               |
|------|---------------------------------|---------------|
| 226. | <i>Ozonium corceum</i> Pers.    | Dale (1914).  |
| 227. | " <i>chrysospermum</i> (?)      | Dale (1914).  |
| 228. | <i>Rhizoctonia solani</i> Kuehn | Pratt (1918). |



# THE RELATION BETWEEN pH AND THE REACTION OF AQUEOUS SOLUTIONS AT VARIOUS TEMPERATURES

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It is common practice to interpret pH values of aqueous solutions, particularly those used in biological work, in terms of the reaction of the medium; that is, a given pH is said to represent an "acid," a "neutral" or an "alkaline" medium. The pH of water at the given temperature is taken as the reference of "neutrality" or the neutral point is attained when  $\text{pH} = \text{pOH}$ . At temperatures from  $18^\circ$  to  $25^\circ$  the pH of water is very nearly 7.0, hence a  $\text{pH} > 7.0$  represents an alkaline and a  $\text{pH} < 7.0$  represents an acid solution.

It is the purpose of this paper to emphasize the change in the pH of water with temperature and to show that this change is sufficiently great to render misleading a too promiscuous use of 7.0 as "neutral" thru temperature ranges common to biological work. A quantitative method will be proposed for the interpretation of pH in aqueous solution in terms of reaction at any temperature.

In the following table are given values for the pH of water at various temperatures as determined by Lorenz and Böhi (1909). These values are used because they were obtained potentiometrically, as are most values of pH in the laboratory, and also because they most nearly represent the hydrogen ion "activities."

The experimental values are given in the first column, while those in column (b) were calculated by Lorenz and Böhi by the use of the van't Hoff isochore. The figures in the last column were calculated by the following empirical equation derived by the author, i. e.,

$$\log (\text{pH} - 5.215) = 0.3450 - 0.00422 t^\circ.$$

TABLE I. VALUES OF THE  
pH OF WATER AS DETER-  
MINED BY LORENZ AND  
BÖHI (1909).

$t^\circ$	pH Exp. (a)	Calc. (b)	Calc. (c)
0	7.432	7.432	7.428
5	.....	.....	7.323
10	.....	.....	7.223
15	7.071	7.071	7.073
20	.....	.....	7.128
25	6.959	6.951	7.038
30	6.880	6.866	6.869
35	.....	.....	6.790
40	6.703	6.714	6.716
45	.....	.....	6.644
50	6.529	6.576	6.576
55	.....	.....	6.512
60	6.450	6.450	6.450
65	.....	.....	6.392
70	6.336	6.336	6.336
75	.....	.....	6.283
80	6.228	6.234	6.232
85	.....	.....	6.184
90	6.137	6.141	6.138
95	.....	.....	6.094
99	6.071	6.066	6.061
100	.....	.....	6.053

It is at once obvious that the change in neutral pH is so great with change in temperature that it cannot be ignored through the ranges of temperature commonly used in the biological laboratory. Two significant statements may then be made:

1. A given pH may represent an acid, neutral or alkaline medium depending upon the temperature.

2. A given reaction of a solution or medium is represented by a different pH for each temperature.

A few examples will illustrate the importance of the above statements. The optimum pH for the action of a certain enzyme is

found to be 6.7 at 30° representing a slightly acid medium. If this pH were used at 50°, at which temperature the neutral pH = 6.576, the medium will be alkaline. Suppose it were found that the values for the optimum pH for a given enzyme at various temperatures are identical with those given in table I. There would be two correct statements of results, the optimum pH decreases with rise in temperature, or, the most favorable reaction of the medium, in this case neutral, is independent of the temperature. If the shift of neutral pH with temperature is not taken into consideration and the value of 7 is used through a considerable temperature range, it would be falsely stated in the above instance that at low temperatures the most favorable reaction of the medium is alkaline, while the most favorable action takes place in a medium of increasing acidity with rise in temperature.

It would seem then that whenever pH is to be interpreted in terms of the reaction of the medium it will be necessary to use the value of neutral pH appropriate for the temperature under consideration. As a quantitative standardization the following procedure is suggested.

Henderson (1917) defines the reaction of an aqueous solution as follows:

$$\text{neutral, } \frac{(\overset{+}{\text{H}})}{(\overset{-}{\text{OH}})} = 1$$

$$\text{acid, } \frac{(\overset{+}{\text{H}})}{(\overset{-}{\text{OH}})} > 1 > \frac{(\overset{-}{\text{OH}})}{(\overset{+}{\text{H}})}$$

$$\text{alkaline, } \frac{(\overset{+}{\text{H}})}{(\overset{-}{\text{OH}})} < 1 < \frac{(\overset{-}{\text{OH}})}{(\overset{+}{\text{H}})}$$

that is, the reaction of the medium is determined by the ratio of hydrogen ion concentration to the concentration of the hydroxyl ion, or let,

$$(1) \quad A = \text{acidity} = \frac{(\overset{+}{\text{H}})}{(\overset{-}{\text{OH}})}$$

$$(2) \quad \text{at neutrality } \frac{(\overset{+}{\text{H}})}{(\overset{-}{\text{OH}})} = 1 \text{ or } A = 1 = N$$

$$(3) \quad \log \frac{(\overset{+}{\text{H}})}{(\overset{-}{\text{OH}})} = \log A \text{ from equation (1)}$$

or

$$(4) \quad \text{pH} - \text{pOH} = \text{pA}$$

Let pN = neutral pH at any temperature, then

$$(5) \quad \text{pOH} = 2\text{pN} - \text{pH}$$

and

$$(6) \quad \text{pA} = 2\text{pH} - 2\text{pN}$$

On the scale above suggested,

$$\text{Neutral, } \frac{(\overset{+}{\text{H}})}{(\overset{-}{\text{OH}})} = 1$$

$$\text{pA} = 0$$

$$\text{Acid, } \frac{(\overset{+}{\text{H}})}{(\overline{\text{OH}})} > 1 \quad pA = \text{negative}$$

$$\text{Alkaline, } \frac{(\overset{+}{\text{H}})}{(\overline{\text{OH}})} < 1 \quad pA = \text{positive}$$

It will occur to the reader that this function  $pA$  is similar to the  $X_H$  proposed by Wherry (1919). However, the above author arbitrarily chose 7.0 as neutral reference and proposed the new scale of  $X_H$  as a substitute for  $pH$ . It seems highly undesirable to the author of this paper that the  $pA$  scale should in any wise be generally used for the expression of  $pH$  values, but that its use should be limited strictly to instances where the  $pH$  values are to be translated into terms of the reaction of the medium. In all  $pH$  studies, however, the temperature used should be stated.

It will often be necessary to calculate the value of  $pH$  for one temperature ( $t^\circ_2$ ) that is equivalent in "acidity" to the  $pH$  at another temperature ( $t^\circ_1$ ). This may be accomplished as follows:

$$(7) \quad 2pH = pA + 2pN \text{ from equation (6)}$$

Let  $pH_1$ ,  $pH_2$  and  $pN_1$ ,  $pN_2$  be values for the respective functions of  $t^\circ_1$  and  $t^\circ_2$ .

$$(8) \quad pH_2 = pN_2 - pN_1 + pH_1$$

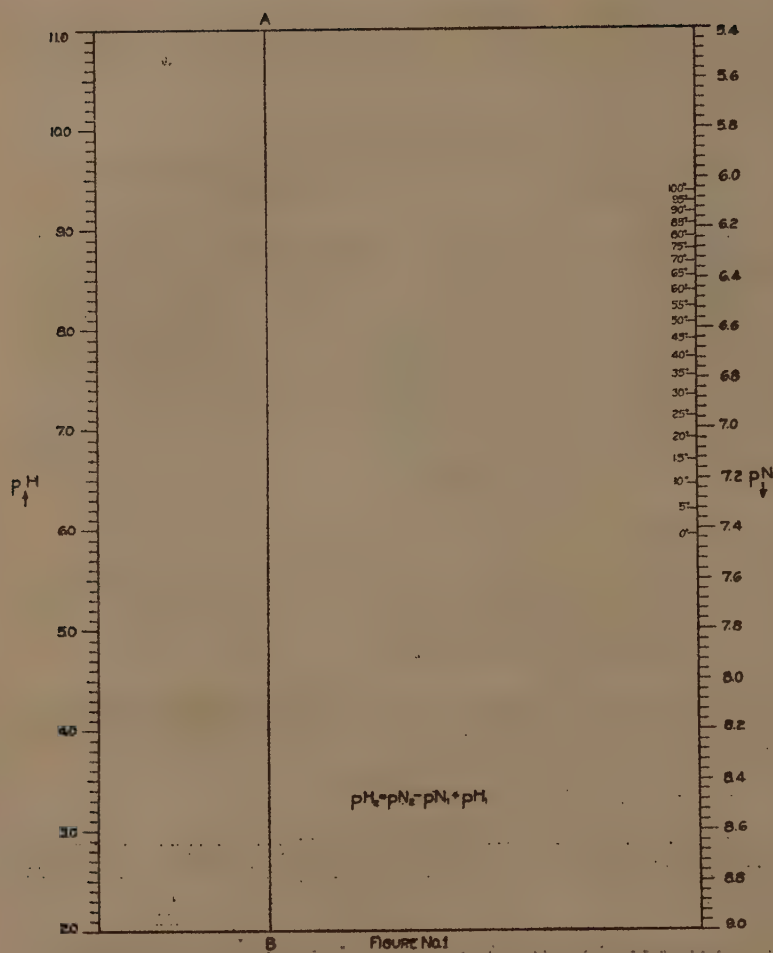
For example, it is desired to know at what  $pH$  a solution must be at  $50^\circ$  to have the same acidity as a solution of  $pH = 7.00$  at  $10^\circ$ . By the use of equation (8) it follows that:

$$pH (50^\circ) = 6.58 - 22 + 7.00 = 6.36$$

That is, a solution whose  $pH = 6.36$  at  $50^\circ$  has the same "acidity" as a solution of  $pH = 7.00$  at  $10^\circ$ . By use of equation (6) it is found that

$$\text{for the solutions discussed above } pA = -0.44 \text{ and } A = \frac{(\overset{+}{\text{H}})}{(\overline{\text{OH}})} = 2.75.$$

In figure I is presented a nomogram by means of which may be calculated the  $pH$  at any temperature which is equivalent in acidity to the  $pH$  at the given temperature. The nomogram takes advantage of the fact that the lines connecting equivalent values of  $pH_1 - pN_1$  and  $pH_2 - pN_2$  intersect on the line  $AB$ . For example, it is desired to know the  $pH$  at  $50^\circ$  ( $pH_2$ ) which represents the same "acidity" as a  $pH$  of 7.00 ( $pH_1$ ) at  $10^\circ$ . Determine the point of intersection on the line  $AB$  of the line connecting  $pH = 7.00$  and the proper value of "neutrality" ( $pN_1$ ) for  $10^\circ$ .



Nomogram for calculation of the pH at any temperature which is equivalent in acidity to the pH at the given temperature.



Construct a line thru this intersection from the proper value of "neutrality" for 50° (pN<sub>2</sub>). The intersection of this line on the pH scale will give the value desired, in this case pH<sub>2</sub> = 6.36.

### SUMMARY

1. Whenever values of pH are to be interpreted in terms of the reaction of the medium at various temperatures, the shift of neutral pH with temperature must be taken into account.

2. A given pH may represent an acid, neutral, or alkaline medium depending upon the temperature.

3. A given reaction of a medium is represented by a different pH for each temperature.

4. Quantitative methods have been proposed for dealing with the above points.

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# GEOGRAPHICAL VARIATION IN THE *NIGRICORNIS* GROUP OF *OECANTHUS* (ORTHOPTERA)

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THERE has been some disagreement among taxonomists working with the Orthoptera, as to whether the dark marked tree cricket described by F. Walker as *Oecanthus nigricornis* is really a distinct species from the pale one described later by Buetenmüller as *O. quadripunctatus*. The two insects are very similar morphologically, but differ in color characters and are usually separated by differences in the markings on the two proximal antennal segments. Caudell, Houghton (1909), E. M. Walker (1904) and Blatchley (1920) have maintained that they are only varieties, that numerous intergrades occur, and that it is impossible definitely to separate the two.

In working with these insects in New York state, P. J. Parrott (1914) and the writer became convinced that they were two distinct species. We found a difference in the choice of host plants for oviposition which was associated with the ecological distribution of the two species. *O. nigricornis* was found in berry plantings and on tall rank weeds or brushy growths of more woody plants such as willow, elder, sumac and grape. *O. quadripunctatus* was found only in old fields, especially where the wild carrot (*Daucus carota*) was abundant. This plant was preferred above all others for oviposition, but occasionally other pithy weeds one to two feet high were used, such as aster, small species of *Solidago*, and *Ambrosia artemisiifolia*.

These habit differences were associated with color characters, the most constant of which was the color of the abdominal sternites, which is difficult to determine in pinned specimens due to the shriveling and discoloration of the body on drying. We also found a constant difference in the morphology of the eggs of the two species.

Rehn & Hebard (1916), studying these insects in the southeastern states, believed that the two species were distinct. They state that in addition to coloration, *O. nigricornis* may be distinguished from the other by "the heavier pronotum, the greatest width of which more closely approximates the length of the same than in that species (*O. quadripunctatus*), while the head between the eyes is weakly but distinctly depressed, a condition not at all or rarely very weakly indicated in *quadripunctatus*." In the specimens examined by the writer, few of which were from the southeastern states, the depression between the eyes has not proven distinct or constant enough to be of any aid in separating the species. In specimens from Ohio and Iowa the pronotum of *nigricornis* averages slightly broader in proportion and also slightly larger in relation to the wings, but here again the difference is slight and the maximum for *quadripunctatus* overlaps the minimum for *nigricornis*.

When the writer moved to Oregon he found tree cricket eggs in berry canes deposited in a manner similar to *O. nigricornis* in the east. They proved to be the eggs of a physiological variety of the snowy tree cricket,

*O. niveus*, an account of which has been published (Fulton (1925)). The former species did not occur there, nor did *quadripunctatus*, but one very similar to both was found abundant in prairie regions where it was closely associated with a certain weed with sticky glandular hairs, called the gum plant (*Grindelia*). This insect had oviposition habits more like *O. quadripunctatus*, but the markings on the proximal antennal segments were very heavy and the ventral segments and sides of the abdomen were only slightly darkened, if at all. The species agreed with Saussure's description of *O. argentinus* and this identification was sustained by Caudell, who stated that he regarded it as merely a variety of *nigricornis*.

The problem of the relationship and distribution of the three species, or subspecies, at once suggested itself. From time to time the writer had opportunity to collect series in various parts of the country and to examine specimens loaned by other collectors.

The problem resolved itself into, first, an attempt to determine the western limits of *nigricornis* and *quadripunctatus* and the eastern limits of *argentinus*, and second, to find out if there is any blending between any or all of the species in question.

## METHODS

In order to facilitate the tabulation of data, it was found advisable to originate a series of arbitrary classes based on the markings of the proximal antennal segments. At first, attempts were made to classify the material in a straight line series from light to dark markings, but it soon became evident that this would not work. It was found that dark body color is rather closely associated with certain types of antennal pattern, and that those with a heavy pattern diverge in two directions from a middle ground, one toward typical *nigricornis* with increasing body color and the other toward typical *argentinus*, without essential increase in body color.

After a number of changes 12 classes were decided on, which seemed to have approximately equal value and served well enough to show the differences in the tree cricket population of various regions with regard to this set of characters. Records were also kept on the degree and extent of dark body color, where this was possible. The frail integument of the abdomen makes records on coloration of that part untrustworthy except in fresh or liquid preserved material.

The 12 classes were based mainly on the markings of the first antennal segment. Numerous records showed that the degree of coloration of the second segment was closely correlated in the majority of specimens. The material was therefore classified on the basis of the pattern of the first segment alone except in doubtful cases. The 12 classes are illustrated in table II, and are described as follows:

1. With no markings.
2. With either the inner or outer elements of the pattern absent and the other reduced.
3. Space between the spot and line at least three times the average width of the line.
4. Space 2 times, but less than 3 times, the width of the line.
5. Space about the width of the line or a little over.
6. Space about one-half the width of the line or a little over. Space of fairly uniform breadth or the narrowest part proximal. Line quite heavy and of nearly uniform breadth.



7. Space distinctly less than one-half the width of the line, and narrowest proximad, or spot and line barely contiguous, leaving a notch distad. Line very heavy and of nearly uniform breadth or narrower distad. Little or no infuscation of the remainder of the segment.
8. Spot and line distinctly confluent; line very heavy. Little or no infuscation of the remainder of segment.
- 6'. Space about one-half the average width of the line or a little over, narrowest part distad. Line not unusually heavy and broadest distad. Segment often slightly shaded distad and proximad of the spot.
- 7'. Space distinctly less than one-half the width of the line, narrowest distad, or spot and line barely contiguous distad. Remainder of segment more or less shaded.
- 8'. Spot and line distinctly confluent, but usually leaving a notch of ground color proximad. Segment distinctly infuscated; palest areas bordering the line on both sides.
- 9'. Pattern decidedly obscured by a general dark infuscation of at least the distal two-thirds of the segment.

In scoring series of specimens on the above plan it is impossible to eliminate the personal element in the interpretation of doubtful specimens. It must be admitted that in series containing many atypical forms, no subsequent examinations would place all specimens in exactly the same classes again. The method serves to give a general idea of the complexion of a series, but conclusions must not be drawn too closely. This work was not intended as a study in evolution or heredity, but is merely an attempt to clear up a taxonomic problem. Better conclusions could be drawn from series which numbered in thousands, but the study has been limited to such collections as were available and to limited opportunity for travel. For several reasons these insects are not ideal material for a special study of variation.

The writer is fully aware that there is such a thing as "place variation," that a species varies somewhat in character from generation to generation or over longer periods of time. Only a few of these series were taken at the same place during different years. There the differences found are probably not greater than would be obtained by the law of chance if the second collection had been made the following day. Field observations on these insects covering seven years in New York and five years in Oregon have convinced the writer that they did not vary noticeably during that time in the characters used for separating the species.

## RESULTS

The results of scoring all material examined are summarized in two tables. In table I are shown the actual numbers of individuals of each class; in table II the percent of each class is shown graphically only for the larger series. In many of these the number of individuals is much too small for drawing any quantitative conclusions. In several cases nothing is known about the ecological conditions where collections were made. In some series, especially those showing two distinct modes, the height of each mode depends entirely on the amount of collecting done in each of the two kinds of habitats, and not on the relative abundance of the two forms in that particular locality.

Other specimens examined fell in the above classes as follows: Mineral Springs, Ind., 3 in class 4; Montreat, N. C., 1 in class 9; Pascagoula, Miss., 2 in class 4; Grant Co., Okla., 1 in class 7; Bozeman, Mont., 2 in class 5;

TABLE I.

Series	Locality	No. of specimens of each class. Upper rows refer to upper series 6' to 9'.									6-6' intermediate	Total
		1	2	3	4	5	6'	7'	8'	9'		
1	Geneva, N. Y. various dates				2	5		7	1			15
2	Newark, Ohio Aug. 25, Sept. 5, 1921			2	11	5		3	23	10		54
3	Gainesville and Newberry, Fla., July-Nov.			5	10	2						17
4	Oskaloosa, Iowa, Sept. 4, 1924				11	23		2		1		37
5	ditto				18	33	1	7				59
6	ditto				6	10	1	2	3	1		23
7	ditto					1	1	10	15	6		33
8	ditto total of 4, 5, 6 and 7				35	67	2	21	18	7	1	152
9	Ames, Iowa Aug. 13, '23			1	20	33						54
10	Ames, Iowa, Aug. 27, '24			2	21	49	1	1				74
11	Ames, Iowa, Aug. 28, '24			1	4	17						22
12	Ames, Iowa Aug. 13, '24					14	14	6	1		2	43
13	Ames, Iowa Aug. 27, '24					1	14	8	2	1	3	29
14	Ames, Iowa Aug. 26, '24				1	9	6	4	1		1	23
15	Ames, Iowa Aug. 28, '24				1	1	5	17	8	1		33
16	Ames, Iowa Aug. 24, '24				19	26	3	4	1			53
17	Ames, Iowa Aug. 13, '24				1	8	7	1				19
18	Ames, Iowa Aug. 26, '24					8	4	3				15
19	Ames, Iowa Oct. 10, '24				1	11	4	3			6	25
20	Ames, Iowa, total of 9 to 18			4	68	177	58 9	47	13	2	12	390
21	Sioux City, Iowa Aug. 16, '25				13	30	4 1	15 7	1 10		2	83
22	Lake Okoboji, Iowa Aug. 18, '25			1		2	3 1	5 1	2 1			15
23	Brookings, S. D. Sept. 18, '23			1	2	6	4 1	10	4		1	29
24	Brookings, S. D. various dates			1	1	2	8 1	12	1			26
25	Lake Hendricks, S. D., Aug. 17, '23				1	1		6 3	3			14

TABLE I—Continued.

Series	Locality	No. of specimens of each class. Upper rows refer to upper series 6' or 9'.									6-6' intermediate	Total
							6'	7'	8'	9'		
		1	2	3	4	5	6	7	8			
26	Arco, Minn., 1911							5				5
27	Canton, S. D. Aug. 26-28, '23		1	3	3	6	3	5				26
28	Yankton, S. D. Sept. 27, '23				7	8	2	1				18
29	Volin, S. D. Aug. 29, '23				2	3	1					6
30	Eastern, S. D., total of 23 to 29		1	5	16	26	15	39	8		1	124
31	Capa, S. D. various dates			3	1	2	2	3	1			14
32	Pierre, S. D. Sept. 10, '21			1	1	1						3
33	Martin, S. D. Sept. 3, '23	9	30	1				6	9			55
34	Aweme, Manitoba			3	2	9	8	8	2		3	35
35	Bismarck, N. D. Sept. 13, '21			5	5	7	4	6	7	5	2	41
36	Whitewood, S. D. Sept. 9, '23					4	1	2	6	2		15
37	Kansas 36 localities		1		3	2	5	44	31			88
38	Galveston, Texas May			1		3						4
39	Brownsville, Texas June					6	10	1				17
40	Fort Collins, Colo. Aug. 16, '23				4	15	1	32	25			77
41	Mesa Verde, Colo. Aug. 14, '24				1	8	2					11
42	Montezuma Co., Colo.				2	10	5					17
43	Wray, Colo.						1		2	2		5
44	Williams, Ariz. Aug. 14, '21					3	5					8
45	Arizona, other localities				2			5	1			8
46	Big Timber, Mont. Aug. 12, '25		1	2	31	46	2					82
47	Boise, Idaho		1	7	12	6	1					27
48	La Grande, Ore. Aug. 16, '19					1	4	9	2			16
49	Corvallis, Ore. Aug. 24, 28, '19						1	21	9			31
50	Corvallis, Ore. Sept. 6, '23						2	26	3			31

TABLE I—Continued.

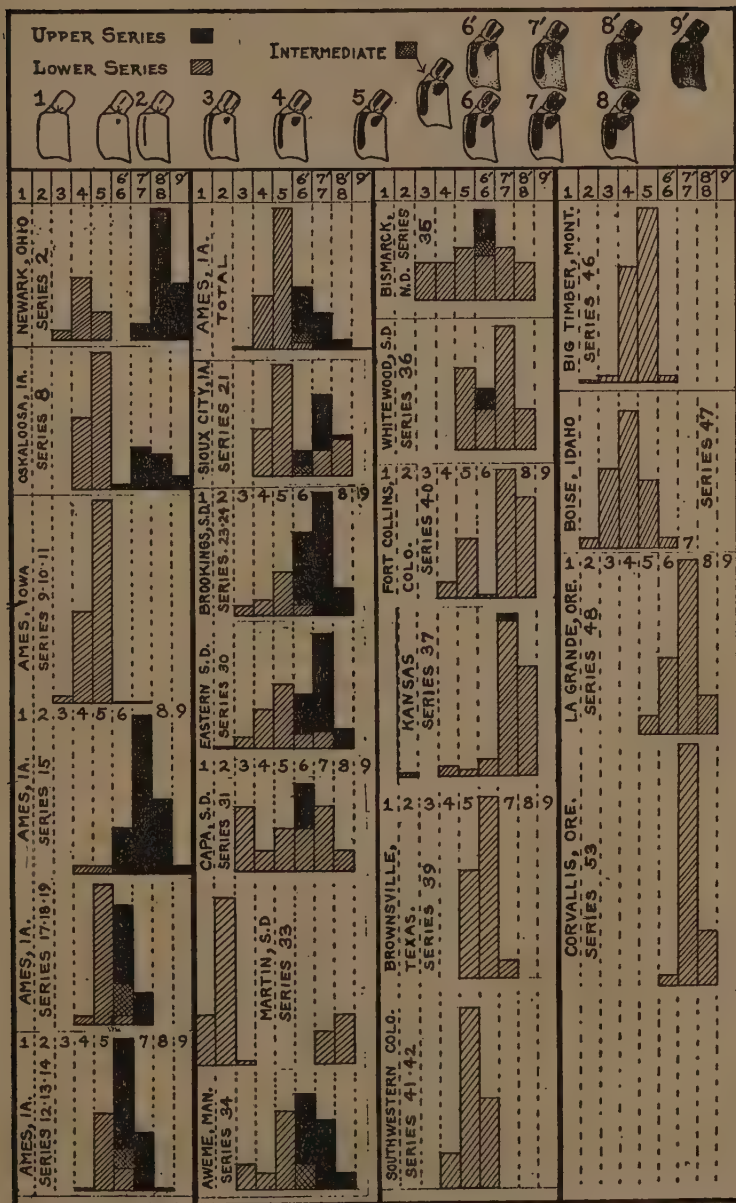
Series	Locality	No. of specimens of each class. Upper rows refer to upper series 6' to 9'.								6-6' inter-mediate	Total
						6'	7'	8'	9'		
		1	2	3	4	5	6	7	8		
51	Corvallis, Ore. Sept. 2, '23						1	15	3		19
52	Corvallis, Ore. Other specimens							25	5		30
53	Corvallis, Ore. Total of 49 to 52						4	87	20		111
54	Medford, Ore. Aug. 3, '21							4	2		6
55	Forest Grove, Ore. Oct., 1906							2	1		3

Billings, Mont., 3 in class 4; Tempe, Ariz., a bottle of specimens collected by R. M. Wilson from alfalfa were examined several years ago and notes taken at the time show that they all had the heavy antennal markings and would fall in class 7 or 8.

Further data on the series of specimens from which data was taken: (2) Newark, Ohio, from *Ambrosia trifida*, river bottom land, and *Daucus carota*, upland fields. (3) Gainesville and Newberry, Fla., collected by Fred Walker, the Newberry specimens from dog fennel. (4) Oskaloosa, Iowa, from *Ambrosia artemisiifolia* on side and top of hill pasture, growing in clumps. (5) from *Solidago* clumps in the same pasture. (6) from *Polygonum* and *Bidens* in a wet gully, same field as last. (7) from tall *Solidago* and *Ambrosia trifida* along fence surrounding above field. (9) Ames, Iowa, from *Ambrosia artemisiifolia* and other small weeds, river terrace pasture. (10) same place, following year. (11) from *A. artemisiifolia*, another river terrace pasture 5 miles from (9). (12) from *Cannabis sativa* and *Vernonia*, creek bottom land, one-quarter mile from (9). (13) same place following year. (14) from same plants two miles down stream from (12). (15) from *Ambrosia trifida* and *Helianthus* along fence half mile from (11). (16) from various kinds of weeds on terrace above creek. (17), (18), (19) three collections from same raspberry planting. (21) Sioux City, Iowa, mainly from *Erigeron canadensis* and *Helianthus tuberosus*, all from same field. (22) Lake Okoboji, Iowa, from various weeds in low meadows adjacent to lake. (23) to (33) and (36) loaned by H. C. Severin. (23), (24), (25), Brookings and Lake Hendricks, S. D., low prairie. (27), (28) Canton and Yankton, S. D., valley land with natural tree growth. (29) Volin, prairie. (31) (32), Capa and Pierre, S. D., central part, latter in Missouri Valley, former in a branch valley 35 miles southwest. (33) Martin, S. D., sand hill area with sparse vegetation. (34) Aweme, Manitoba, loaned by E. R. Buckell, collected by N. Criddle. (35) Bismarck, N. D., mainly from *Grindelia*, Missouri river bottom land. (36) Whitewood, S. D., in Black Hills altitude 3,700; pine, spruce and oak forest region. (37) Kansas, from University of Kansas collection, a few specimens each from many localities scattered over entire state. (38), (39) collected by Snow, University of Kansas collection. (40) Fort Collins, Colo., mostly from tall weeds in low ground, those in classes (4) and (5) from sage brush. (41), (42), (43), collected by C. J. Drake and H. H. Knight. (44) Williams,



TABLE II.



Ariz., from *Chrysothamnus*. (45) Arizona, several isolated records, mostly from University of Kansas collection. Localities given: S. Ariz. (2 in class 4), Bill Wm's. Fork (1 in class 8), Cochise Co., San Rita Mtns., Douglas, Tucson, Phoenix. (46) Big Timber, Mont., from *Grindelia* and *Helianthus*, collected by J. R. Parker. (47) Boise, Ida., loaned by Claude Wakeland. (48) La Grande, from weeds in mountain side gully and field in valley several miles north. (49)-(53) Corvallis, Ore., mainly from *Grindelia*. (49) prairie near creek. (50) dry fields at foot of hill. (51) hillside prairie, mostly on *Hypericum*. (52) miscellaneous specimens, all from prairie. (54) Medford, Ore., from *Grindelia* along railroad.

#### DISCUSSION OF RESULTS

An examination of the tables shows three general conditions in regard to the populations of these species, corresponding largely to three general regions, the eastern deciduous forest region, the great plains and the Rocky Mountain-Pacific Coast region.

In the eastern part of the country, extending a little west of the Mississippi, *nigricornis* and *quadripunctatus* seem to be fairly distinct, sufficiently so that one would be justified in calling them separate species. A few individuals may be found which would be difficult to place on the basis of antennal markings alone, but with fresh material or specimens in liquid, showing clearly the body color, especially the abdominal sternites (fig. I, A. B.), I believe practically all specimens could be properly identified. In this region the two crickets live in different environmental conditions.

By long experience in collecting these insects, the writer can tell at a glance which cricket would be found in any particular plant association in this region. *O. nigricornis* is found among the large, coarse, herbaceous plants with pithy stems or brushy growths of more woody plants or young trees of certain species. *Quadripunctatus* lives only among smaller species of pithy stemmed herbs. This well defined ecological distribution seems to be based on the oviposition habits which may be tested out in cages. If each species is given a choice of a number of different kinds of stems for oviposition, *nigricornis* will select large stems of about 6 to 10 mm. or even

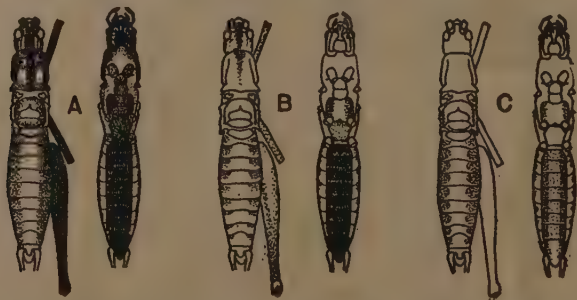


Fig. 1. Showing the extent of melanic color on the dorsal and ventral sides of the body with wings and legs removed except the right femora in the dorsal view. (A) Dark and (B) light colored specimens of *O. nigricornis* from eastern states. (C) Dark form of *O. argentinus* found in some western localities.

larger, while *quadripunctatus* will choose stems of 3 to 4 mm., or seldom as large as 5 mm. If given only one kind of plant, as raspberry for example, *nigricornis* will oviposit in the main part of the cane, while *quadripunctatus* will use the tips or leaf petioles, neither of which would be normal for the latter species.

At Oskaloosa, Iowa, where the two crickets are not as widely separated as they are further east, collections were made in an old pasture extending over a low hill. The fence along the bottom of the hill was surrounded by tall weeds, such as the giant ragweed (*Ambrosia trifida*) and a large species of golden-rod (*Solidago*). In the field, extending over the hill, there were many clumps of somewhat smaller golden-rod and the common ragweed (*Ambrosia artemisiifolia*). Series (4), collected only from the last named plant, contained only 3 specimens of *nigricornis* out of 37, and series (5), taken only from the clumps of golden-rod, contained 8 of the latter species out of 59. Some of these plants were of such size that the lower stems would come within the normal size range for oviposition by *nigricornis*. Series (6), collected from smartweed (*Polygonum*) and Spanish needle (*Bidens*), in a wet gully on the side of the hill, contained a considerable proportion of both forms, but none of doubtful taxonomic position. Series (7), taken from tall weeds along the lower fence, contained only one *quadripunctatus* out of 33. In all the specimens there was close correlation between the type of antennal markings and body color. All in classes (7') and (8') had black or very dark abdominal sternites and most of them were shaded on the sides of the abdomen, and had the typical dark areas on head and pronotum, as well as dark legs and antennae (Fig. I, A. B.). Two of the specimens in (6') showed only faint color on the abdomen, the other was typically dark. These results show that at this locality, which is near the western limit of originally extensive deciduous forests, the two forms are fairly distinct both taxonomically and ecologically.

Series collected in the territory included in the great plains area are more difficult to interpret. This includes territory which is largely prairie, but which may have forest in the immediate vicinity of the larger streams. Here we find all three forms present, *nigricornis*, *quadripunctatus* and *argentinus*, and there are also more atypical specimens which are difficult to place. At Ames, Iowa, which is at about the eastern edge of this area, *argentinus* does not appear to be present, and the other two approach each other more closely and are more often associated than at Oskaloosa, 70 miles to the southeast. Series (11) shows a pure population of *quadripunctatus* taken on common ragweed (*Ambrosia artemisiifolia*) and vervain (*Verbena* sp.), all small plants, 1 to 2 feet high, growing at the top of a river terrace in an old pasture. Series (9) and (10) were taken on two successive years on the side and top of another terrace much closer to the stream, from the same plants as above with the addition of a small golden-rod. In series (10) only one typical specimen of *nigricornis* out of a total of 74 was found and that at the foot of the terrace. Compare these with series (15) taken from giant ragweed (*Ambrosia trifida*) and a *Helianthus* growing along a fence on level ground not over half a mile back from the terrace where series (11) was collected. Here we have only two specimens of *quadripunctatus* out of 33. No such pure populations are found in any of the other series taken around Ames, including those from creek bottom land collected largely from hemp (*Cannabis sativa*) and iron weed (*Vernonia*),

both large plants. The *nigricornis* present falls largely in the paler class (6'). There are many individuals which closely approach typical *argentinus* and fall in class (6) and many which seem to be intermediate in position between (6) and (6').

All of the *quadripunctatus* in series (9), (10) and (11) have entirely pale bodies, while all of the *nigricornis* in series (15) have dark venters, but the typical dark areas of the head and pronotum are faint or narrow. In the other series there is a general correlation between antennal pattern and body coloration, but there are many exceptions. Many in the 6' class show no dark color on the venter, some of the 7' class are only faintly colored, while on the other hand many in class 5 and even a few in class 4 have a more or less dark colored venter. Specimens from the bottom land and berries are also larger on the average than the typical *quadripunctatus* from the river terraces.

At Sioux City, Iowa, which is at the western edge of the state and within the strictly prairie region, all three forms were found in the same field both on tall plants (*Helianthus*) and on small weeds (*Erigeron canadensis*). Series (21) was collected in a field on the side of a small valley which was fringed with trees, but not extensively wooded. There were some intermediate forms, but on the whole the three forms were more distinct than would be expected when finding them so closely associated. As can be noted in the percentage graph in table II, the modes come in classes 5, 7' and 8. Class 6 was almost absent, while class 6' and intermediate forms were few in number. There was close correlation between antennal pattern and body color. All in class 7' had dark venters. One rather doubtfully assigned to 8' had only a faintly colored venter. One in 6' was entirely pale, while another was faintly shaded. One specimen in 4 had a faintly shaded venter.

At Lake Okoboji, in northwestern Iowa, the three forms were also found associated in low meadow land adjoining the lake. An examination of the several series from the eastern part of South Dakota shows that *nigricornis* and *quadripunctatus* predominate in that region as in Iowa, but that *argentinus* is present as a minor element of the population or at least certain individuals show definite *argentinus* tendencies. Much the same condition is found in the series from Aweme, Manitoba. The correlation of body color in the last locality was poor like that in the Ames series taken from berries.

In series (35), collected on the Missouri bottom lands at Bismarck, N. D., we find a fairly uniform distribution of specimens in all classes from 3 to 8, with a minor element in the population showing distinct *nigricornis* characters. None of those in 6' have a very pronounced dark body color and could not be called typical *nigricornis*. A small series from Capa, S. D., (No. 31) shows the same condition, and series (36) from Whitewood, S. D., in the Black Hills, contains only one specimen similar to the above. The Kansas series contains only two specimens of typical *nigricornis*, from the northeastern part of the state near the Kansas river.

Typical *argentinus* forms the largest element in the Kansas series, taken from all parts of the state, a few specimens from each of 36 localities. It also predominates in the Whitewood, S. D., and Fort Collins, Colo., series, both from the western portion of the great plains region. At Fort Collins, the specimens in classes 4 and 5 were taken from semi-desert plants,



largely *Chrysothamnus*, while the typical *argentinus* came from a patch of marsh ground where the crickets were living on tall rank weeds.

Series (33), from the sand hills at Martin, S. D., contained 15 typical *argentinus* and 40 specimens of what I take to be an extreme form of *quadripunctatus*. These were different from any other series I have seen in having the antennal pattern extremely reduced. Some other series have a few individuals with one element of the pattern absent, but in the Martin series this was the mode and nine specimens had no trace of antennal pattern. It is not known whether the two groups were associated in nature or not.

Series (38) from Brownsville, Texas, contained many specimens in which the spot of the antennal pattern was reduced relatively more than the line. These fell in class 5, but many of them were not typical of *quadripunctatus*. There were relatively few typical *argentinus* and most of the specimens were somewhat intermediate between the two, with many irregularities in form of pattern.

In comparing the series from the great plains area we find extreme variation in the complexion of the tree cricket populations even in localities not far removed from each other. Most of them show that the three forms are quite close together and field notes show that in some cases they are associated in nature. While in most cases the typical forms outnumber the intermediate forms, there are enough of the latter to indicate hybridization or blending. *Nigricornis* becomes scarce toward the west and loses its typical coloration, *argentinus* extends eastward almost if not entirely to the limits of the true prairie region. *Quadripunctatus* ranges westward as an insect of the higher and dryer prairie and finally becomes an inhabitant



Fig. 2. Map showing the antennal pattern of specimens from various localities, or the commonest types of pattern found in series of specimens.

of the semi-desert. Whether these last are true *quadripunctatus* seems doubtful on the evidence of the egg characters as brought out later.

In the Rocky Mountain-Pacific region, the classes in the *nigricornis* group are entirely absent and classes with light antennal pattern extend only thru the Rocky Mountains, as indicated by available collections. No series of any size from this region shows a bimodal grouping of the population, but all fall into a fairly normal curve. In specimens from south-western Colorado and Williams, Arizona, and two specimens in the Kansas University collection labelled S. Arizona, there is a peculiar reduction of the spot of the antennal pattern and frequently it is divided, leaving a small portion isolated on the extreme distal part of the first segment on the outer edge. In many of these specimens the line is as heavy as in typical *argentinus*. The few specimens examined from various other parts of Arizona (45) were mainly typical *argentinus*. I also have notes taken before the present study was started, on a series of specimens collected by R. M. Wilson on alfalfa at Tempe, Arizona. These were typical *argentinus* as shown by sketches made at the time. The abdomen was marked by lightly infuscated lines extending across all of the abdominal sternites on each side and by rows of lightly infuscated patches in a dorso-lateral position. These specimens must have been almost identical to average specimens from western Oregon, where the same body markings are present. (Fig. 1, C.)

Series (47) from Boise, Idaho, appears to be typical *quadripunctatus*. These were collected by Wakeland among weeds along irrigation ditches and the collector states that they sometimes oviposit in the twigs of prune trees, a habit which reminds us of *nigricornis* in the eastern states.

Series (46), Big Timber, Mont., collected from *Grindelia* and *Helianthus*, falls mainly in the *quadripunctatus* groups, but shows decided tendencies toward *argentinus*. Many specimens have the lightly infuscated stripes on the venter, and many have the reduced spot common in the south-western states. The first named host plant is the favorite of typical *argentinus* in western Oregon. All series from western Oregon (49 to 55) show remarkable uniformity, with nearly all specimens falling in classes 7 and 8. Series (48) from La Grande, in eastern Oregon, has the same mode, but a larger proportion of specimens show a lighter antennal pattern.

#### EGG CHARACTERS

In studying the tree crickets in New York state it was found that the eggs of *quadripunctatus* could be distinguished from those of *nigricornis* by the structure of the cap at the cephalic end. The cap is a white covering delicately moulded into numerous minute ridges and projections which are arranged in spiral rows in two directions, similar to the scales of a pine cone. The projections gradually increase in length from the base of the cap, reaching a maximum near the apex and decrease again at the immediate apex. Subsequent descriptions of projections apply to those of maximum length unless otherwise stated. *O. nigricornis* egg caps were found to be broadly rounded and considerably broader than long, and the projections were short. In *quadripunctatus* eggs the cap tended toward a conical shape and averaged only a little broader than long, while the projections were finger-shaped and about twice as long as in the other species.

Comparing the eggs from a number of localities we find that they differ more or less in character in each place. (Fig. 3.) At Newark, Ohio,

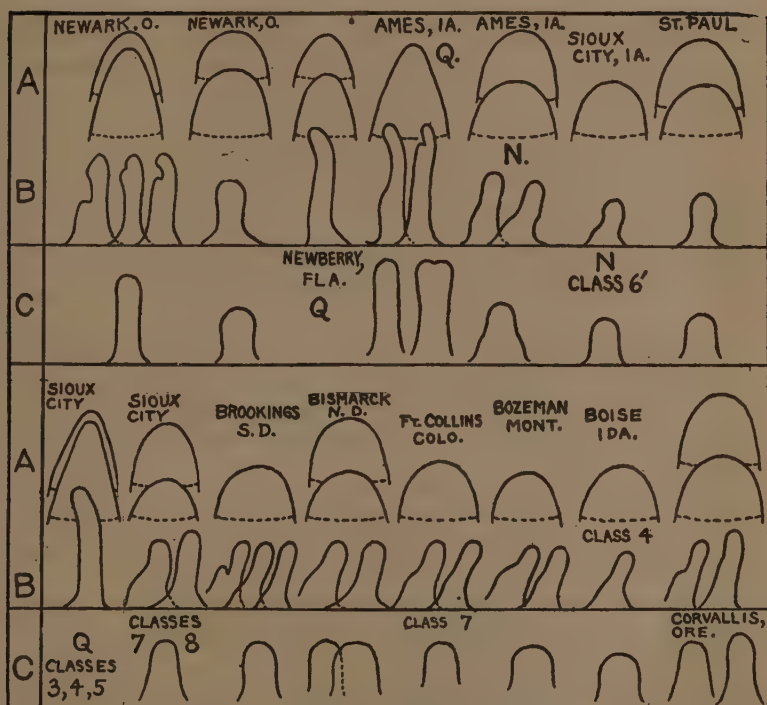


Fig. 3. Eggs of tree crickets. Vertical columns show details of eggs from one locality. (N) *O. nigricornis*. (Q) *O. quadripunctatus*. (Under Newark, O., left column is *O. quadripunctatus*; right, *O. nigricornis*.) When eggs were dissected from specimens the class of antennal pattern is given. In other cases, the eggs were taken from plants. Horizontal rows show: (A) Egg caps, with extremes figured in cases where considerable variation was found. (B) Types of longest projections found as seen at the edge of the cap. The right side is toward the apex of the cap in every case. (C) The same when viewed directly in the middle of the cap. Enlarged 25 diameters for caps, 400 diameters for projections.

the eggs of both species agree closely with the descriptions and drawings made of eggs of the same species at Geneva, N. Y., the only difference noted was that the eggs of *quadripunctatus* from Ohio showed a more pronounced conical shape of the cap and in the majority of them the length of the cap was greater than the diameter. Altho there is the same relative difference in length of cap projections in the two species, they are longer for both species in the Ohio eggs. This difference may possibly be accounted for by an improvement in the method of measuring them.

The projections of the Ohio *nigricornis* eggs are expanded a little near the tip, forming a rounded knob, and are slightly flattened so that the diameter measured on a transverse tangent with the egg surface is a little greater than the radial diameter. A few of the projections, especially toward the base of the cap, have a slight shoulder or buttress on the side toward the base, but this is not the rule. In the eggs of *quadripunctatus*

the projections are also knobbed and slightly flattened at the tip, and usually slightly curved near the tip. Many of them, possibly half, have a more or less abrupt shoulder of variable height. In some cases this forms a ridge or rib extending to the tip.

Eggs collected at Ames, Iowa, on the sites where series (11) and (15) were collected, show even greater differences between the two forms. The *nigricornis* eggs are very similar to those from Ohio, but the *quadripunctatus* eggs exhibit a longer and more conical shaped cap, while the projections are longer, more slender, less clubbed and most of them are provided with a shoulder or buttress.

Eggs collected in wild raspberry canes at St. Paul, Minn., and some from Brookings, S. D., in raspberry (8.5 mm. diameter), and a pithy weed stem (7 mm. diameter), are very similar to the *nigricornis* eggs found elsewhere.

Eggs found in *Grindelia* (5-8 mm. diameter) and wild rose (3 mm. diameter) at Bismarck, N. D., at the same time and place that series (35) was collected, are more like *nigricornis* eggs, altho the oviposition is more like that of *quadripunctatus*. The cap projections differ from *nigricornis* eggs in being broader and in the presence of a low shoulder or buttress on the majority of them.

Eggs collected at Bozeman, Mont., resembled those from Bismarck, but with a more pronounced and more constant development of the shoulder on the projections. At the time these eggs were collected, only two crickets could be found, both of which were placed in class 5, which is the commonest class in the Big Timber series (No. 46).

Eggs from Corvallis, Oregon, have a short rounded cap, but the projections are quite long and resemble those of *quadripunctatus*. Many of them have a shoulder, but probably most of the longer ones do not.

In order to obtain still more information on the egg characters, the dissected eggs from specimens in liquid and also some pinned ones were studied. In most of the series the females did not contain any well developed eggs if collected before September. Oskaloosa, Iowa, specimens showed the same type of eggs for *nigricornis* and *quadripunctatus* as found at Ames. Eggs were obtained from the following specimens of series (21), Sioux City: 1 from class 3, 4 from class 4, 5 from class 5, 2 from class 7, 4 from class 8, 1 from class 6'. Unfortunately, all female specimens in classes 7' and 8' were young and contained no eggs. All eggs from specimens in classes 3, 4 and 5 had the long conical cap with the long projections as at Ames. All other eggs had a short, rounded cap. The projections on eggs from classes 7 and 8 were slender, of medium length, many of them with a slight shoulder, and showed points of resemblance both to Corvallis, Oregon, eggs and to eggs from Bozeman, Mont., and Bismarck, N. D. Curiously enough, eggs from the one specimen of class 6' had the short, stout projections typical of *nigricornis* from Ohio.

Dissected eggs from crickets collected on raspberry at Ames all had the short, rounded cap with short projections. A specimen of class 5 from the creek bottom land, series (12), also had eggs of this type. Most of the Ames specimens were collected before eggs were developed.

One specimen of *quadripunctatus* from Newberry, Fla., contained four well developed eggs. The cap was not excessively long and conical as in



Iowa, but resembled more the Geneva, N. Y., condition. The projections, however, were long and slender, as in Iowa specimens.

Eggs dissected from a dried specimen of class 4 from Boise, Idaho, had the short, rounded cap and short projections. The same was true of eggs from a Fort Collins, Colo., class 7 specimen, and also from a class 5 specimen, but in the latter case the projections were obscured.

The evidence from the egg characters indicates that the tree crickets found on raspberries and on tall bottom land weeds at Ames, Iowa, are *nigricornis* whether the antennal pattern is lighter than the typical condition or not. It indicates also that those with the light antennal pattern from Colorado and Idaho are not true *quadripunctatus*. The egg characters must be fairly constant for the latter species in the east, where the same general type is found in such widely separated localities as New York, Ohio, Iowa and Florida. At Sioux City, Iowa, the meager data seem to indicate that *quadripunctatus* is distinct there also, yet it seems improbable that it could exist without hybridizing when intimately associated with such closely related forms.

The egg characters point toward a close relationship between *nigricornis* and *argentinus*. *Nigricornis* eggs from Ames closely resemble those dissected from *argentinus* specimens from Sioux City, Iowa, and Fort Collins, Colorado.

### CONCLUSIONS

The extent to which local environmental conditions may change the character of the tree crickets of the *nigricornis* group has not been determined. It seems hardly possible that all the results could be accounted for in this way. The environmental difference between a small ragweed growing in a pasture and a bramble or sunflower ten feet away, is hardly great enough to account for the differences in color, egg structure and habits, found between *nigricornis* and *quadripunctatus*. If environment were directly responsible for the differences, why should all three forms be found in the same field and on the same species of plants as at Sioux City, Iowa? Likewise in western Oregon, apparently similar differences in environment have little or no effect on the color characters, which hold remarkably constant.

On the other hand, we find in the far west little or no difference in the egg characters between series which fall largely in the pale classes of antennal pattern and those which are typical *argentinus*. Considering the extreme environmental differences that may be found in the western mountain regions, it seems entirely possible that color characters could be influenced to some extent at least by the physical environment.

On the whole, one is forced to the conclusion that in each locality the crickets have a certain genetic constitution differing more or less from those in other localities. In the eastern portion of the United States and Canada, *nigricornis* and *quadripunctatus* are fairly distinct, sufficiently so to be considered different species if they did not range beyond this region. Both forms are found in most of the eastern territory, but *nigricornis* ranges farther north and reaches the southern states only in the Appalachian Mountain regions. In the eastern portion of the great plains area, *nigricornis* becomes less distinct, by reduction of the average antennal pattern and body color, altho the eggs retain essentially the same character. In



this region it apparently blends or hybridizes with *argentinus*, which has eggs very similar to *nigricornis*. *Nigricornis* predominates in the eastern portion of the area and fades out to the west, while the reverse is true of *argentinus*, which also extends to the Pacific coast states.

The situation in regard to *quadripunctatus* is more uncertain. All known eggs of this form from the eastern states, so far examined, have shown distinct characters, even as far west as Sioux City, Iowa. No eggs were obtained of the extreme form found in the sand hills at Martin, S. D., but it seems plausible that this might represent the extreme western extension of true *quadripunctatus*. The few eggs obtained from apparently typical *quadripunctatus* from Colorado and Idaho were not like those found in the eastern states, but were more like the eggs of *argentinus*.

This evidence points to a closer relationship to *argentinus*. Either the two forms are less distinct in the far west or else true *quadripunctatus* extends only as far as the eastern portion of the great plains, and similar forms found in the far west are only variations of *argentinus*, possibly due to local environment. From a practical standpoint, the best we can do at present is to consider all series with the light antennal pattern and pale body color as *quadripunctatus*.

Until better characters for separating the tree crickets of this group have been discovered, it seems advisable to consider *nigricornis*, *quadripunctatus* and *argentinus* as subspecies. The greatest difficulty in identifying them will be found in the central portions of the country. There they exhibit little or no differences in the choice of environment. One would hardly expect such closely related races to be associated on the same plants without hybridizing to some extent. There is no experimental evidence to show whether this is the case or not, but many individuals are found which are intermediate in regard to the characters commonly used in separating them.

#### OTHER SPECIES

The only other known species of *Oecanthus* in the *nigricornis* group is *O. pini* Beutenmuller, which has characters distinct enough to justify its rank as a separate species. The antennal pattern is not very distinctive. It is usually like that of class 4 or 5, with the spot on the first segment small and round or with an outward extension along the distal border of the segment. The lines on the second segment are straight, parallel, of uniform width and usually well separated. In some specimens the outer elements of the pattern are faint or absent.

The most distinctive character about the species is the general ground color of the body. It could be confused only with specimens of related species discolored by too long confinement in the killing bottle. In dry specimens, it is a light yellowish or reddish brown, lighter on the lateral portions of the pronotal disk and ventral side of the thorax, and darkest on the median part of head and pronotum. The pale color is close to clay color, cinnamon, or cinnamon buff in Ridgway's "Color Standards and Nomenclature," while the darkest parts are near snuff brown, walnut brown, or burnt umber. The arrangement of the darker color is similar to typical *nigricornis*, but in the latter the dark color on the pronotum is fus-cous or black on a pale buff or ivory yellow ground color. The venter of the abdomen is black in typical *nigricornis* as compared to dull brown in

*pini*. The first antennal segment is broader in *pini*, being nearly as wide as long; the ground color of the two basal segments is the same as the palest body color, while the remainder of the antenna assumes the darkest body color.

In life *pini* is easily recognized by the distinct green of the wing veins. The hind femora and dorsal part of the abdomen have enough green to give them an olive color. The head and thorax appear more reddish-brown in life and contrast sharply to the greenish color of the wings. Nymphs have greenish wing pads, a green abdomen with cream colored dorso-lateral stripes; the remainder of the body is light brown; the legs a light olive brown. The species has been found only on or near pines.

~~From the following~~

#### KEY TO NORTH AMERICAN OECANTHINAE\*

On the basis of this and other recent work on the tree crickets, the following modified key is appended:

- A. Front face of the proximal antennal segment with a small but prominent tubercle or knob on the distal border near the middle; no black markings. Hind tibiae armed with spurs only. Wings exceed the tegmina by at least half their length. Deciduous forest and thickets. Conn. to Ga., west to Iowa, Kansas, Texas. Recorded by Saussure from Mexico and Central America.

*Neoxabea bipunctata* (De Geer)

- AA. Proximal antennal segment without a prominent tubercle on the distal border. Distal half of hind tibiae armed with several long spines and numerous small teeth.

#### *Oecanthus*

- B. Front face of proximal antennal segment with a broad, white or ivory colored swelling at the inner edge, ornamented with black. Area between eyes usually tinged with yellow.

- C. Swelling with a round or oval, black (or rarely brown) spot. Second segment with a similar spot. Width of dorsal field of male tegmina nearly half of length. Deciduous trees and shrubs. Me., Ont., and B. C., to Ga., Cuba., Calif., Mexico and Central America.

*O. niveus* (De Geer)

- CC. Swelling with a curved or "J-shaped" black mark; the proximal end curved toward the inner side. Second segment with elongated black mark. Width of dorsal field of male tegmina less than four-tenths of length; length 10 to 12 mm. Pronotum usually with a darker median streak. Deciduous trees and shrubs. Mass., Mich., Minn., S. D., to Fla., Kans., and Tex.

*O. angustipennis* Fitch

- CCC. Swelling with a club-shaped black mark, broadest proximad. Second segment with elongated black mark. Width of dorsal field of male tegmina less than four-tenths of length; length 12.5 to 14 mm. On certain oaks, sometimes on hickory, beech and other deciduous trees. Conn., Long Island and N. C., to Ohio, Tenn., Mo., and Iowa.

*O. exclamatonis* Davis

- BB. Front face of proximal antennal segment without a swelling on the inner edge; this edge nearly straight except at the base and nearly parallel with outer edge.

\* Three other North American species of *Oecanthus* have been described by C. F. Baker. The writer has not examined the types, but in the descriptions there is nothing to definitely separate *O. Rileyi* Baker from *niveus* or *O. rehni* Baker from *argentinus*. *O. marcosensis* Baker is apparently only a dark form of the species commonly accepted as *californicus*.

- C. Subgenital plate of female with a broad notch posteriorly, half as broad as widest part of the plate. Width of dorsal field of male tegmina about half the length (in one case as low as .44). Front side of proximal antennal segment never ornamented with more than a narrow dark line along the inner edge.
- D. Typically pale straw color or ivory, with top of head and base of antennae purplish pink; proximal antennal segment without distinct markings. Large; male tegmina 13 mm. and over; female, 11 mm. and over; ovipositor 6.5 mm. and over. Pronotum width at hind margin seldom greater than length in males, distinctly less in females; pronotum small in relation to tegmina so that width of hind border goes into length of tegmina 5 to 6 times. Hollow on side of terminal segment of maxillary palp rarely covers more than distal third of segment. In thickets of shrubs, vines and tall weeds. Long Island, southern Mich., and Minn., southeastern S. D. to Ga., Miss., and Kans., possibly to Tex.

*O. latipennis* Riley

- DD. Brown to ivory with only a reddish tinge on top of head in pale forms; proximal antennal segment in many specimens with a dark line along the inner edge of front face. Smaller; male tegmina 10 to 13 mm.; female, 8 to 11.5 mm.; ovipositor, 5 to 6 mm. Pronotum of male with width of hind border generally exceeding length by more than one-tenth; in females length seldom exceeds width by more than one-tenth. Pronotum larger in relation to tegmina, width of former included in length of latter 4 to 5 times. Hollow on side of terminal segment of maxillary palp covers distal 3-5 to 1-2 of segment. Thickets of shrubs and other low plants. Wash. (?), Ore., Ida., to Calif., Colo., Ariz., N. Mex., Tex., and Ark. (?).

*O. californicus* Saussure

- CC. Subgenital plate of female with a narrow notch posteriorly, not more than one-fifth of widest part of plate. Male tegmina narrow; width of dorsal field rarely over four-tenths of length. Front side of proximal antennal segment, with few exceptions, ornamented with more than a narrow line along inner edge.
- D. Head, pronotum, legs and antennae light to medium brown, sometimes reddish-brown; underside of abdomen dull brown. In life, wing veins green, dorsum of abdomen and hind femora olive green. In pines or near them. Mass., Conn., Long Island, N. J., Pa., N. C.

*O. pini* Beutenmuller

- DD. Head and pronotum typically with a fuscous median stripe on pale yellowish ground color, (or these parts entirely pale or nearly entirely black). Sternites of abdomen typically entirely black; atypically lightly infuscated or pale. Proximal antennal segment ornamented as in classes 6' to 9', atypically as in 5 (table II). On tall weeds, shrubs, young trees or vines. Typical: Me., Ont., Minn., S. D., to eastern Kans., Mo., Ky., and Va.; in mountains to Tenn. and N. C. Atypical: Man., N. D. to Nebr., western Ia.

*O. nigricornis nigricornis* Walker

- DDD. Body entirely pale or with abdomen lightly infuscated along each side of the sternites and dorso-laterally. Proximal antennal segments ornamented as in classes 6 to 8; atypically with line as in 6, but with spot reduced. Generally on weeds 1 to 3 feet high, or small shrubs. Great plains and west to Pacific.

*O. nigricornis argentinus* Saussure

- DDDD. Body entirely pale. Abdominal venter lightly infuscated in some atypical specimens. Proximal antennal segments ornamented as in classes 1 to 5 (rarely as in 1 and 2). On small weeds 1 to 2 feet high. Typical: Me., southern Ont., Mich., Minn., to Fla., Miss. Atypical: B. C., Man., to Ariz., Tex.

*O. nigricornis quadripunctatus* Beutenmuller

## SUMMARY

Three tree crickets of the genus *Oecanthus*, namely *nigricornis*, *quadripunctatus* and *argentinus*, differ from each other mainly in color characters and have been separated by the nature of the black markings on the two proximal antennal segments. There has been some dispute among taxonomists as to whether they represent distinct species.

In order to classify the material from various localities on the basis of the antennal pattern, twelve approximately equal, arbitrary classes were originated. The classes with light pattern (*quadripunctatus*) run in a straight line series up to a middle point and from there diverge in two directions, toward the extreme types of *nigricornis* and *argentinus*.

In the eastern portion of the U. S. corresponding roughly to the country originally largely covered with forest, *nigricornis* and *quadripunctatus* are present and distinct in color characters, ecological distribution and habits, as well as having distinct types of eggs. They could be considered separate species if they did not extend beyond this region.

In the great plains region, *nigricornis*, *quadripunctatus* and *argentinus* are found, with many intermediate forms. The three are often associated in the same field on the same kinds of plants. The characteristics which typify *nigricornis* gradually disappear from the population toward the west, while the reverse is true of *argentinus* characters, which come no farther east than the true prairie regions.

Only *argentinus* and *quadripunctatus* are found in the Rocky Mountain region and are not clearly separated. The former only has been found in the Pacific Coast region.

*Quadripunctatus* has the same distinct type of egg in N. Y., Ohio, Fla. and Iowa. In the western region the eggs of individuals falling in the *quadripunctatus* classes of antennal pattern are of a different type and resemble those of typical *argentinus*.

Until better characters are discovered for separating the three tree crickets of this group, it seems advisable to consider them as subspecies.

A revised key to the *Oecanthinae* of North America is appended, based on this and other recent work.

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# NOMOGRAM FOR DETERMINATION OF GENERATION TIME AND VELOCITY COEFFICIENTS FOR RATES OF GROWTH OR DEATH

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THE value of nomographic or alignment charts has long been recognized in many of the sciences and in their applications. Apparently they have been but little used in biology. It is the purpose of this article to present a nomogram which is useful in the calculation of growth and death rates of microorganisms.

One of the commonly used methods of evaluating the effect of any environmental factor upon an organism is to make comparisons of growth rates or death rates under different conditions. This may be most satisfactorily accomplished during the so-called logarithmic growth phase, and during the analagous logarithmic death phase, when this is found to occur.

In a study of growth rates during the logarithmic growth phase, determinations are usually made of the generation time, or the velocity constant of the rate of growth (rate of growth per cell). These relationships may be expressed mathematically as follows:

$$b = B2^{t/g} \text{ or } b/B = 2^{t/g} \quad (1)$$

$$\text{and } b = Be^{kt} \text{ or } b/B = e^{kt} \quad (2)$$

in which  $B$  = No. of cells (as bacteria) at beginning.

$b$  = No. of cells after time  $t$ .

$g$  = Length of one generation time, expressed in same units as  $t$ .

$e$  = Base of natural logarithms.

$k$  = Rate of growth per cell (Velocity constant of the rate of growth).

Similarly in a study of death rates, if such rates are logarithmic, the following relationships hold:

$$b = B2^{-t/g} \text{ or } B/b = 2^{t/g} \quad (3)$$

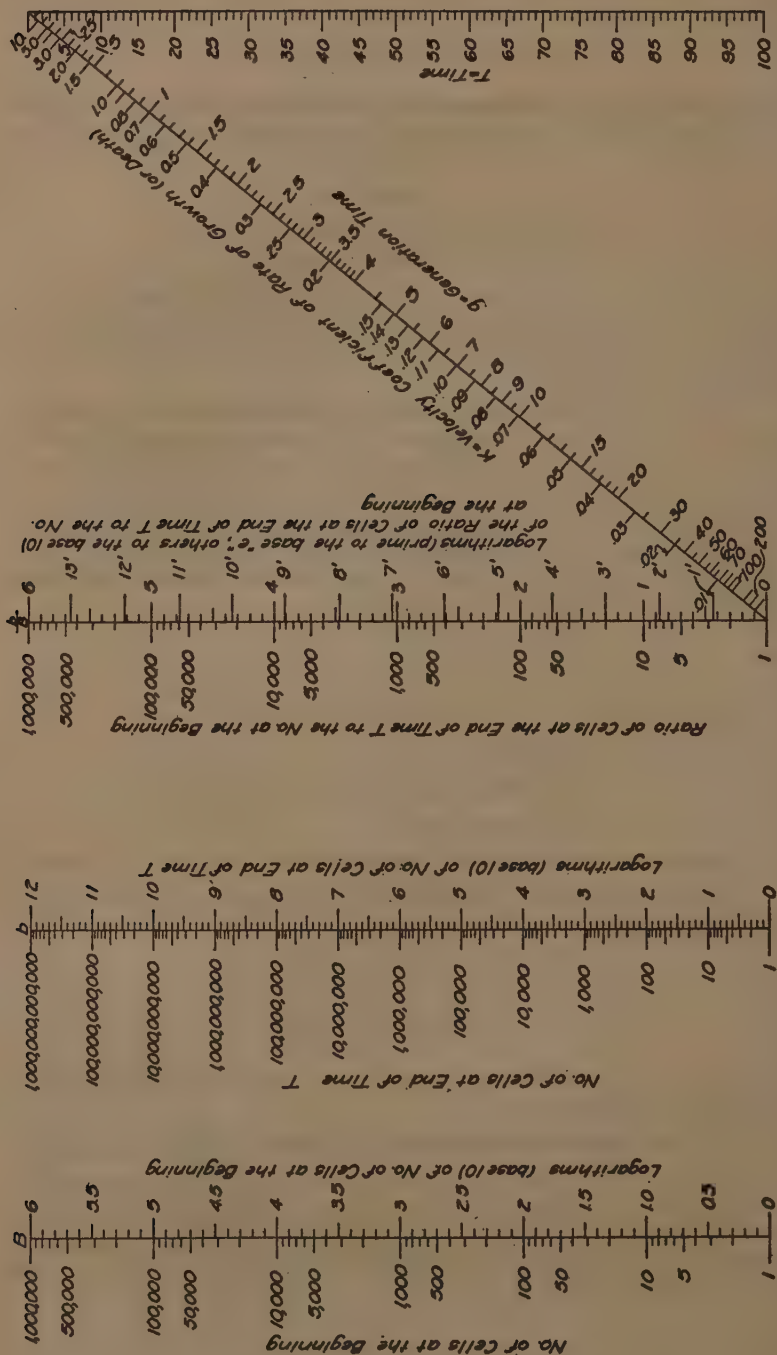
$$\text{and } b = Be^{-kt} \text{ or } B/b = e^{kt} \quad (4)$$

Equations (3) and (4) are similar to (1) and (2), differing in having the number ratios inverted, and

$g$  = Time required for the number of cells to be decreased by one-half.

$k$  = Velocity constant of the rate of death (Rate of death per cell).

From the accompanying nomogram it is possible to determine for any of these equations the value of any one of the four quantities if values are assigned for the other three. Usually the three known are  $b$ ,  $B$  and  $t$ , the one to be evaluated  $g$  or  $k$ .



Nomogram for determination of generation time and velocity coefficients for rates of growth or death.

*a. To determine generation time during logarithmic growth.*

$b = B2^{t/g}$ . Place a straight edge to draw a line through the determined values of  $B$  on Axis 1 and  $b$  on Axis 2. The intersection of this line projected with Axis 3 gives the value of  $b/B$ . Connect by a straight edge this point on Axis 3 and the given value of  $t$  on Axis 5. The intersection with Axis 4 gives the corresponding value for generation time ( $g$ ). Similarly if values are known for any three of the variables, one may determine the other.

*b. To determine rate of growth per cell (velocity constant during logarithmic growth.*

$b = Be^{kt}$ . Proceed as under *a*, except read value of  $k$  from the upper side of Axis 4. It will be noted that Axis 4 constitutes a stationary scale from which values of either  $g$  or  $k$  may be read if the other is known.

*c. To determine the time for half the cells to be killed during logarithmic death.*

$b = B2^{-t/g}$  or  $B/b = 2^{t/g}$ . It will be recalled that equation (3) is the same as equation (1) with  $B/b$  replacing  $b/B$ . The same nomogram is used as for (1) except that  $b$  is read on Axis 1 and  $B$  on Axis 2. The method of *a* is used otherwise.

*d. To determine the velocity constant of the rate of death during logarithmic death.*

$b = Be^{-kt}$  or  $B/b = e^{kt}$ . Follow directions for *b*, except read  $b$  on

Axis 1 and  $B$  on Axis 2.

If  $B$  be defined as initial concentration of a substance undergoing monomolecular change, and  $b$  the concentration after time  $t$ , then this nomogram may be used for determining the velocity constant of the monomolecular reaction.

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NOTE. It will be noted that the nomogram makes provision for 100 units of time only. If time, as in minutes, is greater, use a larger unit. For example, if  $t$  is 300 minutes, use say 10 minutes as the unit of time, and take reading on the Axis 5 at 30. The result secured on Axis 4 should be multiplied by 10 to convert into minutes.



# A STUDY OF CROWN GALL CAUSED BY *PSEUDOMONAS TUMEFACIENS* ON ROSACEOUS HOSTS\*

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THE recent investigations by Riker (1923a), (1923b), and those by Robinson and Walkden (1923), showing that *Pseudomonas tumefaciens* Sm. and Town. probably does not push through the tissues in intrusive tumor strands, have afforded a new angle of attack upon the crown gall problem. In fact, the results from the above researches may be said to have reopened the whole crown gall problem in its relation to rosaceous plants and to the general practices of propagation of apples and many other types of nursery stocks. Such biological aspects of the causal organism as its prevalence in overgrowths and hairy root formations and its longevity in the soil in competition with other bacteria, as well as the normal processes of excess callus formation at the union of piece-root grafted stocks, and their pathological effects upon young and old trees, become questions of major importance.

In this paper consideration will be given only to those aspects of the crown gall problem relating to the presence or absence of *Ps. tumefaciens* in the various types of overgrowths on nursery stock, the development of non-infectious overgrowths and hairy root formations, the possibility of infection resulting from the soil and the possible effect of any type of overgrowth of apple and peach on the water conducting capacity of the tree.

## I. CULTURAL STUDIES OF *PS. TUMEFACIENS* FROM APPLE GALLS AND HAIRY ROOT.

In a study of the effect of overgrowths at the union of piece-root grafted apple trees, discarded by the nurseryman as crown gall, it became at once imperative to determine to what extent *Ps. tumefaciens* was present in these excrescences. Extensive isolations therefore were made not only from such overgrowths but also from trees and seedlings showing various types of hairy root formation. The organisms recovered, identical with or closely resembling *Ps. tumefaciens* in plate cultures, were studied as to their pathogenicity on tomato plants. At the same time consideration was given to the overgrowths and the hairy root formations from the stand-

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\*\* The writer wishes to acknowledge his indebtedness to Dr. I. E. Melhus, under whose direction this work was done, for helpful criticism and suggestions received throughout the course of these investigations.

These studies have been carried out in connection with the crown gall project in which the Crop Protection Institute of the National Research Council, University of Wisconsin, U. S. Department of Agriculture, Office of Mycology and Disease Survey, and Iowa State College are cooperating.



point of gross appearance and texture in their relation to the healing processes incident to propagation by grafting.

#### DESCRIPTION OF TREES EMPLOYED

Representative trees, discarded by the nurserymen as infected with crown gall and those showing the effect of overgrowths on water conduction, were collected and employed in isolation studies. These trees were piece-root grafted, two years old or two-year "cutbacks" showing overgrowths usually at the union. They were selected for the most part by Dr. I. E. Melhus and the writer in 1924 from a lot of Wealthy trees discarded in the field as crown gall by the nurserymen at the time of digging. In other cases similar trees of the varieties Wealthy, Jonathan, Stayman, Delicious and others were culled from nursery stock in storage, either by the writer or nursery employees. Typical specimens of these trees are shown in plate 1, fig. A.

The overgrowths varied in size, shape and extent to which they encircled the tree. In most cases they were located at the union, usually at the tip of the scion lip. In some cases they occurred at the tip of the stock lip and less frequently along the sides of the union.

A large majority of the overgrowths were hard and woody in texture, with a definite bark layer. The exterior of the gall was rough, granular in appearance and showed an excessive development of root primordia, fine fibrous or apparently normal secondary roots. Such overgrowths were typical of those described by Hedgecock (1910) as the "woolly knot" form of hairy root. A second type was small in size, softer in texture, with few woody elements within the body of the overgrowth and without secondary roots, root primordia or a bark layer. A third type less commonly found was smooth surfaced, without roots or root primordia, soft in texture and in some cases somewhat spongy with no bark layer.

In addition to these two-year-old trees, seedlings typical of those discarded in grading as affected with hairy root were secured from a nursery in Kansas in the spring of 1924 and isolations were made. There was no evidence of an overgrowth nor of wounds at the point of origin of the fibrous roots that arose in clusters of three to ten. The development of this type of root clusters occurred on the upper portion of the main root, that is, from the crown to a distance of 1 to 4 inches downward. Such roots appeared to have a normal origin in the pericycle and developed later in the season than the secondary roots of normal seedlings. In some cases these fibrous hairy roots were brown throughout their entire length.

#### METHOD OF ISOLATION AND INOCULATION

In the progress of the isolation studies two methods of procedure were followed, the second of which, developed by M. K. Patel (1926), was a modification of the first to take care of certain difficulties inherent in the isolation of a specific organism from soil-contaminated roots.

In the usual method of isolation (Smith, Brown and Townsend (1911) and Smith (1920) ) the surface of the gall is first disinfected with a 1-1000 solution of mercuric chloride or flamed and small pieces dipped in the disinfectant before crushing. More recently Riker (1923a), Walkden (1921) and Robinson and Walkden (1923) have shown that surface disinfection of the galls with mercuric chloride results in a decided reduction in the

number of viable bacteria in plates made from the crushed tissue. In poured plates made from galls of *Chrysanthemum frutescens* dipped 10 seconds in a 1-1000 mercuric chloride solution, Robinson and Walkden (1923) report the numbers of crown gall bacteria reduced from 200 to 1. An attempt was therefore made to eliminate this source of difficulty in the isolations.

The following method, first employed successfully with galls from tomato, peach and red raspberry, was used thruout the earlier part of the isolation studies. The gall was thoroughly washed in running water with a hand brush to remove the dirt and loose surface tissue. After drying, the surface of the gall was covered with 95 percent alcohol and flamed. Small pieces of the tissue about 1 centimeter square were next removed from two or three places with flamed scalpels, placed in a sterile petri dish and 1 cubic centimeter of sterile water added. The material was finely macerated and allowed to stand for one to several hours to allow the bacteria to diffuse out of the tissue. In case the material was kept for several hours, it was placed in the refrigerator to check the development of chance contaminants. From this suspension, dilution plates were poured, using two three-millimeter loops of inoculum in 10 c.c. of neutral potato dextrose agar. The plates were examined daily and none was discarded before 10 days or more had elapsed. Control plates were poured from galls produced by artificial inoculation on apple, willow or tomato and from naturally infected galls on apple, peach, raspberry or viburnum, previously proven to be caused by the crown gall organism. Colonies resembling *Ps. tumefaciens* in the plates were streaked upon potato dextrose agar slants and later inoculated into tomato.

In several cases the third dilution plate was so heavily sown that it was impossible to identify the crown gall organism. Such plates were flooded with 2 cubic centimeters of sterile water, which made a heavy suspension, and then inoculated into tomato. As a further precautionary measure, the suspension of macerated tissue from the overgrowth employed in making dilution plates was inoculated into healthy tomato plants.

In previous attempts at isolation employing potato dextrose agar, when a large amount of inoculum from the macerated tissue was used, the plates in many instances were overrun with contaminants. When old gall tissue was macerated, molds also developed rapidly in the plates. A modification of the method of isolation was devised in the bacteriological laboratory by M. K. Patel by which large amounts of inoculum could be employed in making the plates with less contamination. Patel's (1926) method consisted essentially of the use of a medium in which sodium taurocholate and crystal violet inhibited the growth of most of the contaminants found in apple gall tissue. A large amount of inoculum, 1 to 2 cubic centimeters, was added to 25 c.c. of the medium and plates poured. The efficiency of this method was first tried by pouring the bile salt agar directly upon macerated apple gall material. In four days typical colonies of *Ps. tumefaciens* had developed, while the bacterial contaminants were almost entirely absent. This method was employed in the later cultural studies upon a lot of 85 trees.

#### ISOLATION STUDIES OF 1925

In these cultural studies, isolations were attempted from overgrowths at the union of 111 piece-root grafted apple trees. These consisted of lots

of the varieties Wealthy, Salome and Jonathan selected at digging time in 1924 from those discarded in the field by the nurserymen or from those in storage after field culling.

The overgrowths on the trees of the different lots presented much the same appearance with respect to size, consistency, location on the union, presence of roots and root primordia and character of the surface. A description of one lot of 25 Wealthy trees will suffice to give a very good idea of the appearance of the entire number. Where exceptions occur in the remainder of the trees, these will be noted in the subsequent discussion. In this typical lot of 25 Wealthy trees, the overgrowth was found at the tip of the scion lip in 14 cases and along the side of the union in four cases. It occurred once at the tip of the stock lip, once at the base of a lateral root from the scion lip and once at the point of a break in the union. In four of the trees the overgrowth appeared to be due to a swollen union, i. e., the stock was much larger in diameter than the scion. Only one gall located at the side of the union showed evidence of decay. In 22 of the trees, fibrous secondary roots arose from the finely convoluted surface of the excrescence. In the other three trees the surface of the gall was smooth without secondary roots or root primordia. In all cases a definite bark layer was present. All the overgrowths were of the hard gall type typical of "woolly knot."

Isolations were made from a lot of 29 Wealthy trees obtained from an Iowa nursery. Plates were poured Jan. 24, 1925, from the suspension of macerated material taken from three places on the overgrowth. At the same time control plates were poured from a soft gall on the root of a two-year-old peach tree and from a gall on tomato. The isolations from all the apple trees were negative. The crown gall organism was recovered from both the peach and tomato galls.

The second lot of 12 piece-root grafted Wealthy trees, received from an Iowa nursery and similar to those in the first series, was employed in the isolation trials on Feb. 1. In 11 of the lot the overgrowths occurred at the union. The remaining one was hard in texture, free of fibrous roots and located at the crown of the tree. Eight of the galls at the union were hard and rather woody, but could be cut to half their depth with a scalpel. However, the deeper tissue was quite woody. There was a definite bark layer and fibrous roots developed in abundance from the malformation. The remaining three galls were relatively soft in texture with a convoluted surface and few fibrous roots. All isolations from this lot of trees were negative. In plates from three galls of the hard, fibrous type, organisms closely resembling *Ps. tumefaciens* were obtained. These colonies in plate culture were circular, wet shining, convex, 1 to 3 millimeters in diameter and appeared after five days. Inoculations with subcultures into tomato were negative. Control isolations from a tomato gall were positive.

Eight two-year-old piece-root grafted Salome trees were selected from those discarded in the field at an Iowa nursery. The galls were hard in texture and the surface appeared finely pebbled due to the development of root primordia. Fibrous roots also developed from the overgrowth. The galls were thoroughly washed in running water, rinsed in sterile water and ground in a food chopper previously sterilized in boiling water. Plates were poured on Feb. 11, 1925, from the material finely macerated in sterile water. While colonies resembling the crown gall organism were found in plates

from each isolation, no infection was obtained upon inoculation into tomato. Control plates from a gall on tomato produced by artificial inoculation yielded the crown gall organism.

Another lot of seven piece-root grafted two-year-old Wealthy trees, selected by Dr. Melhus and the writer from those discarded in the field as affected with crown gall, was employed in making isolations on Feb. 18, 1925. The overgrowths in every case occurred at the union, usually at the tip of the scion lip. These were hard in texture, with an abundance of fibrous roots developing from the overgrowth. Colonies suspected of being *Ps. tumefaciens* developed in plates from three of these trees, but failed to produce crown gall when inoculated into young tomato plants.

Trials were also made on Jan. 28, 1925, from four budded two-year-old Jonathan trees received from a nursery in Missouri. The overgrowths, comparatively soft in texture, with a slightly convoluted surface and no secondary roots, occurred on the stock about 3 inches below the crown. The crown gall organism was not recovered from these galls, although colonies having the appearance of *Ps. tumefaciens* appeared on the plates and were inoculated into young tomato plants.

On Feb. 25 isolations were made from the overgrowths at the union of four piece-root grafted two-year-old Jonathan trees sorted from storage at a Missouri nursery. These overgrowths were small, extending not over one-fourth the distance around the union, with an elevation and diameter of 2 centimeters. The surface of the overgrowths was slightly convoluted, their texture soft. Small fleshy roots arose from the gall. Tissue was taken from the overgrowth after flaming and scraping off the surface. From the four series of isolations, *Ps. tumefaciens* was recovered in one case. In this case the gall was soft in texture with fleshy roots arising in a cluster. The tissue was taken from the gall 2 millimeters below the surface.

A lot of 32 piece-root grafted Jonathan trees two years old was selected by the writer on March 10, 1925, from storage at a Missouri nursery. The overgrowths varied in size and were soft in texture, with a slightly convoluted surface. Fifteen of the galls occurred at the tip of the scion lip, 10 along the side of the union, 4 upon the scion above the union, 1 at the tip of the stock lip, and 1, a secondary gall, arose from an overgrowth at the tip of the scion lip. In one case the overgrowth was merely a swollen union. Seven overgrowths were free of roots; five showed fibrous roots developed from them and 20 were fleshy. Organisms resembling *Ps. tumefaciens* developed on plates from all these overgrowths. Inoculations from subcultures, macerated tissue and suspensions from the plates showing *Ps. tumefaciens*, resulted in the production of a gall on tomato in only two cases. In one case the overgrowth with a few fibrous roots was about one-half inch in diameter and occurred at the side of union. The tissue under the surface from which isolations were made had a slightly greenish tinge as contrasted with the brownish outer layer. The second overgrowth yielding the crown gall organism was a soft gall about 1 centimeter in diameter located at the tip of the scion lip. A cluster of fleshy roots arose from the gall.

Isolations were made from a mixed lot of 15 trees on April 18, 1925. These consisted of 10 Winesap, 2 Jonathan and 3 Wealthy piece-root grafted trees. All of the overgrowths on the Winesap trees occurred at the union, extending from one-fourth to one-half the distance around the tree. Six of



the overgrowths bore fibrous and two bore fleshy roots, while the other two had no roots. The surface of the gall in each case was finely convoluted. Those galls bearing no roots were soft in texture, while those with roots could be classed as "woolly knots." In the Jonathan trees the overgrowths were hard and rough, with an abundance of fibrous roots. One of the Wealthy trees showed a typical woolly knot arising from the tip of the scion lip. In the second tree the overgrowth was granular, flat and bore clusters of fibrous roots. The third tree bore a soft gall without roots on the scion lip. From the Winesap trees, the crown gall organism was recovered in three cases as follows: from a soft gall without roots at the side of the union; from a soft overgrowth with an abundance of fibrous roots arising from the tip of the scion lip; and from a similar gall without roots at the tip of the scion lip. From the Wealthy tree showing the flattened overgrowth bearing clusters of fibrous roots, the crown gall organism was recovered. Isolations from the Jonathan trees were negative.

The results of the isolations from 111 piece-root grafted trees from three nurseries in two states show that *Ps. tumefaciens* was recovered in seven cases.

These results were obtained for the most part from trees discarded by the nurserymen because of overgrowths at the union. No attempt was made to select lots which would yield the pathogen in a high percentage of cases. Further trials were made on one lot of trees grown in soil suspected of being heavily infested with *Ps. tumefaciens*.

This lot of 21 piece-root grafted two-year cutback Wealthy trees, obtained from a Nebraska nursery, was grown on an area from which badly galled roses were dug two years previously. They were selected by Dr. Melhus as suspected crown gall trees. The overgrowths were comparatively large, averaging one and one-half inches in length, two inches in width and one-half inch in elevation. Of this lot 17 showed no fibrous roots from the overgrowth and in 10 of them a bark layer was absent. In these 10 cases the absence of bark was associated with the spongy texture of the overgrowth. The overgrowth in 13 cases occurred at the tip of the scion lip, at the tip of the stock lip in 4 cases and on the stock at a broken union in the remaining 4 cases. Two trees, typical of those yielding the crown gall bacteria, are shown in plate 2, fig. A, while in fig. B are shown two of the same lot in which the overgrowth appeared to be due to excess callus and from which *Ps. tumefaciens* was not recovered.

Isolations from these overgrowths yielded the crown gall organism in 16 cases. Of the galls showing positive isolations, six were hard and ten spongy; in five a bark covering was present and fibrous roots developed from the overgrowth. These results show that from a large number of trees discarded at the nursery because of overgrowths, certain ones can be selected which will yield the crown gall organism in a high percentage of cases.

After this lot of trees had remained in storage five months, re-isolations were made to check the previous results. During this period the storage had become too warm and the overgrowths showed evidence of decay. Isolations were made employing the method as developed by Patel. Of the plates from the 21 isolations, 7 showed molds but no bacterial growth, 3 were sterile, and in 11 small, circular, slightly opaque, white, wet shining colonies developed. From these colonies, suspected of being *Ps. tumefaciens*, inocu-



lations were made into young tomato plants, but no galls developed. Further inoculations were made at the same time from the suspension of macerated tissue and from these galls developed in 15 of the 21 cases. These results indicate strongly an inhibitory action of rot-producing organisms when growing in competition with *Ps. tumefaciens* in a medium suitable to both. It is also evident that failure to obtain *Ps. tumefaciens* in culture from decaying galls is not proof of its absence or non-viability in the overgrowth.

#### ISOLATIONS IN 1926

In the early part of the cultural studies, isolations were made during the period Jan. 24 to April 18 from trees kept in temporary storage. During the latter part of this period storage conditions became very unfavorable. Due to the high temperature and moisture necessary to prevent drying out of the trees, bacteria and molds multiplied rapidly upon the overgrowths and in a few cases decay had set in.

Under these conditions it seemed possible that numbers of the crown gall bacteria, if present, might have been killed off, and therefore larger amounts of inoculum should have been used in making the poured plates. With the method of isolation employed at this time, increasing the amount of inoculum was not feasible because of greater contamination. It is possible that with a method more suited to this condition, a higher percentage of positive results might have been obtained.

A modification of the early method made it possible to use greater quantities of the inoculum with less drastic surface disinfection of the tissue and less chance of contamination.

Employing the method developed by Patel (1926), further isolations were made from 85 cutback trees of several varieties. The first lot, consisting of 15 Wealthy three-year-old cutback trees obtained from an Iowa nursery in 1924, had been set in the experimental plots for one year. These trees, with two exceptions as noted later, were especially selected from our stock of nursery discards in 1925 as showing hard overgrowths typical of excess callus formation. Isolations from these trees, however, had not previously been made to determine the presence of the crown gall organism in the overgrowths. Of the 13 trees the overgrowth occurred at the tip of the scion lip on 3, at the side of the union on 3, on both stock and scion lips on 2, and along the side of the union in 2 cases. The three remaining cases showed rough, swollen unions. The overgrowths on these trees half encircled the tree and averaged one and one-half inches in length, two inches in width and three-fourths inch in elevation. As controls isolations were also made from one apple and one tomato gall produced by artificial inoculation. The crown gall organism was recovered from four of the trees and from both of the controls. Of those trees selected as showing callus overgrowths the three positive for *Ps. tumefaciens* showed a swollen union with a rough, wrinkled surface and few fibrous roots. The texture of the outer portion of the overgrowth was soft, but they were hard and woody within. Only one of the two trees selected as typical of crown gall yielded the pathogen. The galls in both cases were spongy in texture and occurred upon hard, woody overgrowths arising from the scion lip.

Similar trials were made on eight piece-root grafted two-year-old Delicious trees from a Missouri nursery. These trees were selected from stor-

age before the final culling was done. Seven of these showed an abundance of fibrous roots arising from the overgrowth, which averaged 1 inch in length, 1 inch in width and three-quarters inch in elevation. Only one overgrowth was free of fibrous roots. None of these trees yielded the crown gall organism. Bacterial colonies were found in only two of the plates and they did not resemble the crown gall organism. Inoculations from the macerated gall tissue also proved negative. The overgrowth in two cases occurred at the end of the main root and along the side of the union, while in the three remaining cases it had developed at the tip of the scion lip.

Eight one-year-old grafted Rome Beauty trees were obtained from the storage of the same nursery. These were collected by the writer because of the abundant development of fibrous roots from the overgrowths at the union. Four of the trees also showed fleshy roots arising from the gall. In contrast with the other trees employed in isolation trials, these showed only slightly raised overgrowths, which appeared to be callus formations with a finely convoluted surface. The deep tissue was hard and woody. In seven of the trees the overgrowths occurred along the side of the union, while in the other tree it was found at the end of the stock lip.

The crown gall organism was recovered from three overgrowths, all at the side of the union and two bearing fleshy roots. Colonies resembling *Ps. tumefaciens* developed in three other plates, but upon inoculation failed to produce galls on tomato plants.

A lot of 14 two-year-old grafted Stayman Winesap trees were sorted from storage at a Missouri nursery. All these trees bore overgrowths at the union, from which an abundance of fibrous roots developed. Only one overgrowth gave rise to fleshy roots. The galls in eight trees occurred at the tip of the scion lip, in four at the tip of the stock lip, and in two they arose from the side of the union. With the exception of one smooth surfaced gall at the side of the union, all were wrinkled. They varied in size, averaging three-fourths inch in length, one inch in width and one-half inch in elevation from the union. In consistency the large galls were soft in texture to a depth of one-half inch, with the deeper tissues hard and woody. One small gall, measuring one-half inch in diameter and one-fourth inch thick, was soft throughout. Colonies resembling *Ps. tumefaciens* developed in plates from nine of the overgrowths. These were streaked upon potato dextrose agar and inoculations made from subcultures into young tomato plants. Galls developed on the tomato plants inoculated from subcultures from four of the overgrowths. The overgrowths yielding the crown gall organism all developed at the end of the scion lip. One gall was soft in texture throughout, while the other three were soft to a depth of about one-half inch, with the deeper tissue woody. All bore abundant fibrous roots and the surface of the galls was finely convoluted.

A lot of 20 trees of mixed varieties was received from an Iowa nursery. These consisted of Gano, Baldwin, Ben Davis and Wealthy, altho individual trees were not marked with the varietal name. The overgrowths occurred as follows: 11 at the end of the scion lip, 4 along the side of the union, 3 on the stock below the union and 1 each at the base of a lateral root from the scion lip and 3 inches above the union on the scion. Two galls were partially decayed and from five no roots developed. The surfaces of all those showing fibrous roots were slightly wrinkled or presented a granular appearance. None was extremely hard for a depth of one-quarter

inch, but in general the deeper tissues were woody. In two of the trees the overgrowth was flattened, granular and extended along the entire side of the stock lip, from which clusters of fibrous roots arose. These were typical of hairy root as described by Stewart, Rolfs and Hall (1900). Colonies resembling *Ps. tumefaciens* developed in plates from 13 overgrowths. Inoculations into tomato plants resulted in galls in five cases. Similar inoculations from the macerated tissue resulted in gall formation from the same overgrowths, showing positive results for the cultures.

Twenty trees were received from the storage of a Nebraska nursery. These were two-year cutbacks of an unnamed variety (probably Yellow Transparent). In 18 of the trees the galls occurred at the tip of the scion lip and in the other 2 at the end of the stock lip. They were finely granular in appearance, with fibrous roots, except in two cases in which roots were absent. They varied in size from one-half by three-quarters by one-half inches to one and one-half by three-quarters by two inches. In four cases the overgrowth appeared to have developed as a result of breaking the stock in planting the graft. The crown gall organism was recovered from five galls, four of which occurred probably from infection of the wound made in breaking the stock in planting and one at the tip of the scion lip encircling three-fourths of the union. Suspected colonies of *Ps. tumefaciens* developed in seven plates, but inoculations from two of them were negative. Eight of the plates remained sterile after 18 days incubation at room temperature.

The results of the isolations from 85 trees from four nurseries in three states show that the crown gall organism was recovered in 16 cases or from 18.7 percent of the overgrowths.

In summarizing the results of all the trials, employing both methods of isolation, we find that *Ps. tumefaciens* was present on 23 of the 196 overgrowths studied. The results obtained from the lot of 21 trees from a Nebraska nursery showing 76 percent infection are not included, since they were especially selected from known infested soil. In the early studies of 1924 and 1925, a lower percentage of positive isolations was obtained as compared with those of 1926. This can be accounted for to some extent by the difference in varieties of trees employed; variation in weather conditions from year to year affecting heavier soil infestation in certain of the nurseries from which the trees were obtained; and finally, to some extent, the difference in isolation technique employed.

The results obtained from all these studies are in substantial agreement with those of Riker and Keitt (1925a), (1925b), and show that *Ps. tumefaciens* is present in a comparatively small percentage of the trees discarded at the nursery because of overgrowths at the union.

Although as shown by Smith (1911), (1920), *Ps. tumefaciens* is more difficult of isolation than many other plant pathogens, the fact that from one lot of apple trees grown on soil probably infested, the crown gall bacteria were recovered in 76 percent of the trials, suggests the absence of infection in a majority of the 196 overgrowths studied and points to some other factor as the cause of the excrecences. In this connection the following statement of Melhus (1926) seems significant, "that we have in the past confused excess callus, called hard gall, with true crown gall; and non-pathogenic hairy root with pathogenic hairy root cannot be doubted." When one considers all the facts, it seems quite probable that under the variable

conditions in the nursery, the trees showing true crown gall would not reach a greater number than 15 to 20 percent as an average. It is shown that from large numbers of such discards, lots of trees can be selected which show a high percentage of crown gall infection. In the isolation studies, the overgrowths on 155 of the 196 trees bore an abundance of fibrous roots typical of Hedgecock's (1910) woolly knot form of malformation. The crown gall organism was recovered from 16 of the trees showing this type of overgrowth. It is evident from these results that the presence of an abundance of fibrous roots arising from an overgrowth cannot be regarded as evidence of the presence of the crown gall organism.

Since the isolation studies on galled trees showed that the presence of fibrous roots from an overgrowth could not be relied upon as an index of infection by the crown gall organism, it seemed advisable to make similar studies on apple seedlings showing the development of fibrous hairy roots in an effort to determine the connection between this type of abnormality and crown gall.

Isolations were made, therefore, from 50 seedlings, typical of the hairy root condition previously described, employing potato dextrose agar. Tissue, including about 1 millimeter of the fibrous root, was taken from beneath the cluster of roots. At the same time similar isolations were made from single fibrous roots on 23 normal seedlings. From 42 of the hairy root seedlings dilution plates failed to show colonies resembling the crown gall organism. In the remaining eight, a few wet shining, circular, almost translucent colonies appeared. These were streaked and inoculated into young tomato plants. At the same time inoculations were made from the suspensions of crushed tissue used in making the plates. Control isolations were made from crushed tomato galls. None of the hairy root or normal seedlings yielded the crown gall organism, while *Ps. tumefaciens* was recovered in every trial from the infected controls. The results of these trials suggested that the fibrous type of hairy root on seedlings is not a manifestation of crown gall infection. Further data bearing on the cause of this abnormality were obtained under controlled field conditions.

#### THE DEVELOPMENT OF OVERGROWTHS AND HAIRY ROOT IN THE ABSENCE OF *PS. TUMEFACIENS*

The isolation studies on piece-root grafted trees discarded at the nursery because of overgrowths at the union showed first, that in a majority of cases *Ps. tumefaciens* was absent and, second, that the gall commonly occurred at the basal end of the scion or tip of the scion lip. This led to attempts to induce such overgrowths in the absence of the crown gall organism.

#### OVERGROWTHS

First attempts were made to induce overgrowths on scion wood cut under aseptic conditions and stored in steamed soil. A lot of Wealthy scions was disinfected with a solution of mercuric chloride 1-1000 for five minutes, cut into suitable lengths with a flamed knife and the basal ends of the pieces cut as in grafting. These were buried in sterilized sand in the greenhouse in April, 1926. In two weeks time the white callus tissue, finely convoluted in appearance, had developed from the cambial layer. Two to three weeks later an abundance of this tissue had developed, especially at the ex-



treme tip of the cut. At this point a knob of white granular tissue, in some cases as large in diameter as the scion, had formed. This knot had the appearance of small overgrowths frequently found at the tip of the scion lip on one-year grafts in the nursery. Typical specimens of the callused scions about one month old are shown in plate 3, fig. A.

This growth suggested that, in the case of loosely wrapped grafts or those in which the cambial layer of the scion lip cut to a thin tip, extended beyond the cut on the stock, similar overgrowths might be due to excess callus. In order to test this hypothesis a second lot of grafts was made under aseptic conditions using Wealthy scions. This variety was selected because of the high percentage of trees discarded in the nursery showing overgrowths at the union. Before cutting the stocks or scions they were dipped five minutes in a 1-1000 solution of mercuric chloride. The knife was dipped in alcohol and flamed before making each cut. The bench was also disinfected with the mercuric chloride solution.

Two types of grafts were made: 30 in which the stock and scion fitted perfectly and 45 in which the thin tip of the scion lip extended about three-sixteenths of an inch beyond the cut on the stock, exposing the cambium layer at this point. The grafts were stored three months in new shingle tow and planted out of doors in a plot of soil, steamed 45 minutes at 90 pounds pressure.

Six weeks after planting, a wound was made with a flamed scalpel in the scion above the union in the perfectly fitted grafts. At the end of the season, out of 14 growing grafts, 11 had developed a conspicuous overgrowth at the point of wounding. In the other three the wound had healed leaving only a flat scar. Of the second type, 29 grafts grew and in every case showed a well-developed overgrowth at the tip of the scion lip. Trees showing these overgrowths and typical of the lot are shown in plate 3, fig. B.

Isolations were made from five of the wound overgrowths produced on the trees grown from perfectly fitting grafts. No colonies resembling *Ps. tumefaciens* developed on the plates. The plates, however, were flooded and the suspension inoculated into tomato plants. Likewise, suspensions of the macerated tissue from these overgrowths were also inoculated into tomato plants. All inoculations were negative.

In a similar manner isolations were made from the overgrowths arising at the tip of the scion lip in five trees grown from grafts made with the overlapping scion. Inoculations from suspected colonies of the crown gall organisms and from suspensions of the macerated material failed to produce gall, on young tomato plants. At the same time isolations were made from galls on five one-year-old apple trees and from one tomato plant produced by artificial inoculation. Two of the apple galls were partially decayed, but from the other three and from the tomato gall *Ps. tumefaciens* was recovered.

Although only a few grafts were employed in these trials, the results obtained appear significant and are in accord with those reported by Keitt (1925), in which overgrowths were induced at the scion tip of aseptically made scion cuttings and grafts planted in sterilized soil in the greenhouse and also upon two-year-old apple trees by certain types of wounding.

The absence of *Ps. tumefaciens* in cultures from the overgrowths at the union of piece-root grafted trees discarded at the nursery, and the readiness with which similar malformations could be induced under aseptic



conditions on certain varieties such as the Wealthy, coupled with the tendency of these varieties to show similar malformations in the nursery, all point to the conclusion that such excrescences were not entirely due to crown gall infection, but, in many instances, to the development of excess callus incident to the grafting process.

#### HAIRY ROOT DEVELOPMENT IN STEAMED SOIL

Observations in February, 1925, on over ten thousand unsorted seedlings showed that fibrous hairy root was the type of malformation most commonly found. Typical specimens are shown in plate 4, fig. A. The negative results of the isolation studies and the prevalence of the fibrous form of hairy root on seedlings grown under various soil conditions suggested that this type might be the result of environmental conditions or an inherent tendency of the seedling itself, rather than infection by the crown gall organism. An attempt was therefore made to induce the fibrous form of hairy root on apple seedlings in the absence of *Ps. tumefaciens*.

During the season of 1925\* several thousands of seedlings from disinfected seed were grown under field conditions in steamed soil and counts were made at digging time on the amount of hairy root present. In addition, seed similarly treated and untreated was planted in ordinary field soil. The field where the experimental plots were planted had grown apple seedlings in 1890, 1891, 1904 and 1924. A strip of land 10 feet wide and 110 feet long was thoroughly steamed by the inverted pan method, using a pressure of 90 pounds per square inch for 45 minutes. This strip was surrounded by boards 12 inches wide, divided into five plots and planted to French apple seed previously disinfected by dipping in mercuric chloride solution 1-1000 for five minutes. The plots on the steamed soil were treated as follows:

Plot I. The seedlings were wounded just beneath the surface of the soil with a disinfected knife.

Plot II. The seedlings were treated as in plot I, but the wound was smeared with a culture of *Ps. tumefaciens*.

Plot III. Each seedling was wounded as in plot I. The soil was then infested with *Ps. tumefaciens* by pouring nine gallons of a water suspension of the organism along the rows at the base of the stem of the seedlings.

Plot IV. The seedlings were not wounded, but the soil was infested as in plot III.

Plot V. Check. The seedlings were not wounded and the soil was not infested with the crown gall organism.

A strip 2 feet wide was left between the plots in which the seedlings were used as guard rows, but not included in the counts. This separation of the plots seemed safe, since in other experiments on grafts in the open, infection was not communicated from the inoculated to wounded uninoculated grafts six inches apart. In only two cases was an infected seedling found in the guard rows. In these, they were wounded plants within two inches of the inoculated seedlings.

The seedlings were hoed before any soil treatments or inoculations were made. The hoe and the workman's shoes were disinfected with mer-

\* These experiments were carried out at the nursery of J. H. Skinner and Company, Topeka, Kan. The writer wishes to express his thanks for the cooperation which made this work possible.

curic chloride. No further cultivation was given. The infestation of the soil with *Ps. tumefaciens* and inoculation of the seedlings were made on June 29 and the plots harvested on Nov. 11, 1925.

The data obtained from these plots are presented in table I. In this and subsequent tables the term "woolly knot" is employed to designate that form of hairy root associated with true crown gall. The term "fibrous hairy root" designates the production of an abundance of fine roots arising in clusters from any point along the main root in the absence of any swelling or overgrowth.

TABLE I. HAIRY ROOT ON APPLE SEEDLINGS ON INFESTED AND NON-INFESTED STEAMED SOIL.

	Total No.	Clean		Woolly knot form		Fibrous form	
		No.	Percent	No.	Pct.	No.	Pct.
Plot I							
Seedlings wounded, not inoculated ...	360	284	79.89	0	0	76	21.11
Plot II							
Seedlings wounded, inoculated directly	710	555	78.18	65	9.15	90	12.67
Plot III							
Seedlings wounded, soil infested .....	690	616	89.27	4	0.59	70	10.14
Plot IV							
Seedlings not wounded, soil infested..	774	697	90.05	0*	0	77	9.95
Plot V. Check							
Seedlings not wounded nor inoculated, soil not infested .....	1482	1366	92.18	0	0	116	7.82

\* Gall on one plant; no hairy root

### Summary of Table I.

From these data it is evident that in no case was the woolly knot form of hairy root, associated with true crown gall, obtained, except by actual inoculation of the seedlings or infestation of the soil, by *Ps. tumefaciens*. On the other hand, the fibrous type of hairy root was found consistently in all plots, either in the presence or absence of the crown gall organism. There appears to be little increase in the amount of fibrous hairy root in seedlings inoculated with *Ps. tumefaciens* as compared with those not inoculated and grown in non-infested soil. In plot II, 12.6 percent of the inoculated seedlings showed fibrous hairy root, while in plot V, without inoculation or soil infestation, 7.8 percent were affected. The highest percentage of hairy root, 21.1, was shown on the wounded seedlings on non-infested soil in plot I.

In the infested soil plots, III and IV, there was little difference shown in the wounded and unwounded seedlings. The percentage of wounded seedlings affected was 10.1 and of unwounded, 9.9. In plot IV, in which the soil was infested but the seedlings were not wounded, the woolly knot form did not occur, but a gall in the absence of hairy roots was found in one case. It is possible that when the seedlings were hoed, just before infesting the soil, they were wounded or a wound may have resulted from insect injury, thus providing a place of entrance for the crown gall organism. The amount of the woolly knot form of hairy root in plot II also indicated that the seedling roots were not readily infected under the field conditions of 1925, even by direct inoculation into a wound.

On the east side of the steamed soil plots there were five rows of seedlings in unsteamed field soil, grown from French seed disinfected with

mercuric chloride solution. The seedlings in two rows, plot VI, were wounded, while in the other three rows, plot VII, they were unwounded. The soil was not artificially infested with *Ps. tumefaciens*. The data from these plots are presented in table II.

TABLE II. HAIRY ROOT ON SEEDLINGS FROM DISINFECTED SEED ON UNSTEAMED SOIL.

	Total No.	Clean		Woolly knot form		Fibrous form	
		No.	Pct.	No.	Pct.	No.	Pct.
Plot VI Seedlings wounded, soil not infested..	327	269	82.26	8	2.45	50	15.29
Plot VII Seedlings not wounded, nor soil infested .....	624	575	92.14	4	0.64	45	7.22

As shown in table II, the woolly knot form of hairy root was found both on wounded and unwounded seedlings in unsteamed soil. This soil had previously grown four crops of seedlings, the last one in 1924. In the wounded, 2.4 percent, and in the unwounded seedlings, 0.64 percent were affected with the woolly knot form of hairy root. Thus by wounding, four times as many seedlings were affected. On the west side of the steamed soil plots were 20 rows of seedlings grown from French seed. The seed was not disinfected and the unsteamed soil previously had grown four crops of seedlings.

The soil and seedling treatments in this plot were made June 29, 1925, as follows:

Plot VIII. Seedlings wounded and soil immediately infested with *Ps. tumefaciens*.

Plot IX. Seedlings not wounded and soil infested.

Plot X. Seedlings wounded, soil not infested.

Plot XI. Check. Seedlings not wounded and the soil not infested.

The seedlings were examined and counts made on the numbers affected with hairy root. These data are presented in table III.

TABLE III. HAIRY ROOT ON SEEDLINGS FROM NON-DISINFECTED SEED ON UNSTEAMED SOIL.

	Total No.	Clean		Woolly knot form		Fibrous form	
		No.	Pct.	No.	Pct.	No.	Pct.
Plot VIII Seedlings wounded, soil infested .....	405	361	89.13	1	0.24	43	10.63
Plot IX Seedlings not wounded, soil infested	344	321	93.31	1	0.29	22	6.40
Plot X Seedlings wounded, soil not infested	1226	1133	92.41	3	0.24	90	7.35
Plot XI. Check Seedlings not wounded, soil not infested .....	1127	1019	90.43	3	0.26	105	9.31

The results obtained in plots VIII and IX show that little infection took place on the wounded seedlings where the soil was infested immediately thereafter by applying a suspension of the crown gall bacteria. This was in contrast with the results, given in table I, where the direct inoculation into the wounded seedlings on plot II gave approximately 9 percent infection.

It is also seen that the amount of infection on unwounded seedlings in infested soil was quite negligible. The percentage of the fibrous type

of hairy root varied little for the different plots. If we concede that this soil was already uniformly infested from the four previous seedling crops, the comparatively constant percentage of the fibrous form of hairy root found in the plots artificially as well as naturally infested indicates that there was probably no relation between this type of abnormality and crown gall infection.

The development of the woolly knot form of hairy root, almost entirely from direct inoculation of the wounded seedlings, indicated that this form is an infection. The occurrence of the fibrous form under all conditions of the experiments also indicated that this form of hairy root was not an infection and may be the result of environmental conditions or some inherent character of the seedling.

#### LONGEVITY OF *PS. TUMEFACIENS* IN THE SOIL

Cultural studies of galled apple trees taken from a field in which severely infected roses had been dug two years previously indicated that the crown gall organism lived over in the soil for a considerable period of time. Riker (1922) states that the crown gall organism retains its viability after a year in sterilized soil. Reddick and Stewart (1924) have shown that *Ps. tumefaciens* remains viable in moist sterilized loam and quartz for a period of 195 days. With the exception of a brief note (Muneie (1926b)) so far as the writer is aware, no data are available on the longevity of the organism in unsterilized soil. An attempt was made, therefore, to determine how long the organism might remain viable and retain its virulence in the soil, in the absence of susceptible host parts and in competition with other soil organisms.

#### Methods

A series of 6-inch pots filled with unsterilized composted greenhouse soil was infested with a four-days-old culture of *Ps. tumefaciens*. The inoculum was prepared by diluting 50 c.c. of peptone dextrose culture to one liter with sterile distilled water. After thoroughly moistening the soil, 80 c.c. of the bacterial suspension were poured upon it. The pots of infested soil were allowed to stand two hours, then lightly watered to insure the penetration of the bacteria into the soil.

Succulent young tomato plants, 6 inches in height were transplanted, after the soil was washed from the roots, into the infested soil. Inoculations were made after the plants had recovered from the effects of transplanting, usually within three or four days.

The following method of inoculation was employed. The soil was dug away from the crown of the plant to a depth of one-half inch and the stem thus exposed was smeared with the infested soil. Needle pricks into the plants were made through the infested soil, which was carried into the wound. The soil was then replaced, insuring sufficient moisture to prevent drying out of the wound. Healthy plants in non-infested soil were similarly wounded as controls. Except when otherwise stated, six plants were grown in infested soil and the same number held as controls in non-infested soil. After conspicuous galls had been formed, the infected plants were removed and the soil thoroughly stirred. Other healthy plants were reset in the pots and inoculated in the same manner.



*Results of Inoculations.*

Two lots of infested soil were employed, one receiving the bacterial suspension Nov. 11, and the other Dec. 21, 1924. Inoculations were made at intervals from Dec. 23 to April 3, 1925, covering a period of 102 days.

The results of the inoculations of infested soil into tomato plants are presented in table IV.

TABLE IV. LONGEVITY OF *PS. TUMEFACIENS* IN NON-STERILIZED SOIL.

Date of soil infestation	Date of inoculation	Number days in soil	Number plants inoculated	Number inoculated plants infected	Number control plants infected
Dec. 21	Dec. 23	2	6	6	6
Dec. 21	Dec. 25	4	6	6	6
Dec. 21	Dec. 30	9	6	0	6 <sup>1</sup>
Dec. 21	Jan. 5	15	6	2	6
Dec. 21	Jan. 22	31	6	0	0 <sup>2</sup>
Dec. 21	Jan. 28	36	6	1	6
Dec. 21	Feb. 4	43	6	1	6
Nov. 11	Dec. 30	48	6	0	6 <sup>3</sup>
Nov. 11	Jan. 5	54	6	6	6
Dec. 21	Mar. 18	86	12	3	6
Dec. 21	Apr. 3	102	12	3	0

<sup>1</sup> All plants rotted at point of inoculation.

<sup>2</sup> Three plants rotted and one dead.

<sup>3</sup> All plants rotted at point of inoculation.

The results of inoculations clearly show that *Ps. tumefaciens* was viable and pathogenic after remaining in non-sterilized soil for a period of 102 days. After four days in the soil there was a decrease in the number of infected plants. This may have been due to smaller numbers of the pathogen in the soil due to leaching, or to the inhibitory effect of the other organisms in the non-sterilized soil. From the above data this cannot be determined. However, it will be noted that rotting occurred at the point of puncturing in all the plants inoculated Dec. 30 and that no galls were formed. In this experiment, the lack of gall development suggests that rot-producing organisms may so affect the host plant that the growth of *Ps. tumefaciens* is inhibited. Similar negative results were obtained from the inoculations of Dec. 30, using soil infested Nov. 11 with the same strain of the organism. Inoculations from this lot of soil on Jan. 5, however, resulted in 100 percent infection, showing that lack of infection in the previous inoculations was not due to the absence of viable, pathogenic, crown gall bacteria.

Robinson and Walkden (1923) have shown that large numbers of *Ps. tumefaciens* occur on the surface of primary galls on *Chrysanthemum frutescens*. It seemed possible that the pathogen might be washed from the tomato galls in watering, thus re-infesting the soil after each series of inoculations and prolonging the apparent period of viability in the soil.

A second series of trials on the longevity of the organism in the soil was made, eliminating this possible source of error.

In these trials, inoculations were made with infested non-sterilized and sterilized soil. The same strain of *Ps. tumefaciens* was employed here as in the previous experiments. Two types of soil were employed, namely, greenhouse compost rich in humus and a medium heavy clay. The soil was infested by thoroughly wetting it with a suspension of the crown gall organism made from 150 c.c. of a three-day-old peptone dextrose culture and an



equal volume of setrile distilled water. The flask of sterilized soil, 64 days after infestation, became contaminated with a mold. This gradually developed until it had spread throughout the soil in the flask. Sterilized and non-sterilized soil not infested was thoroughly wet with sterile water to be used in control inoculations. The compost was infested Jan. 12, 1926, and the clay soil on Feb. 25. Inoculations were made by needle pricks into the succulent parts of the plants for a distance of about one inch along the stem. The inoculum was prepared by making a thin paste of the soil in sterile water. This was then smeared upon the upper portion of the stem and pricked into it with a sterile needle.

In each trial plants were inoculated with the infested and non-infested soils. As a check on the splashing of the pathogen from the inoculated plants, additional healthy plants were pricked with a sterile needle and placed between those inoculated. The results of inoculations into healthy young tomato plants are presented in table V.

TABLE V. RESULTS OF INOCULATION OF INFESTED GREENHOUSE SOIL INTO HEALTHY TOMATO PLANTS.

Date		Number days organism in soil	Number plants inoculated with each soil	Number infected plants from inoculation with:				
Inoculated	Examined			Non-sterilized infested soil	Sterilized infested soil	Non-Sterilized non-infested soil	Sterilized non-infested soil	Sterile needle
1-13	2- 2	1	5	5	5	0	0	0
1-14	2- 2	2	5	5	5	0	0	0
1-18	2-18	6	5	5	5	0	0	0
1-21	2-18	9	5	5	5	0	0	0
2- 3	2-24	22	5	5	5	0	0	0
2-18	3- 6	36	5	5	5	0	0	0
2-24	3-10	43	5	5	5	0	0	0
3- 1	3-22	47	5	5	5	0	0	0
3-11	3-30	58	5	5	5	0	0	0
3-17	4- 2	64	5	5	5*	0	0	0
3-26	4- 6	73	5	5	5	0	0	0
4- 2	4-23	80	5	3	4	0	0	0
4-10	4-29	88	4	4	3	0	0	0
4-20	5-12	98	5	4	2	0	0	0
5- 3	6- 8	110	5	3	0	0	0	0
6-17	7-14	154	5	3	0	0	0	0

\* A mold found growing in the flask of infested sterilized soil on this date.

The results of the inoculations show that *Ps. tumefaciens* remained viable and retained its virulence on tomato after 154 days in non-sterilized greenhouse soil rich in humus.

Sixty-four days after infesting the sterilized soil, the culture became contaminated with a mold. As shown by the results of inoculations, the development of the mold in the culture was associated with a decrease in the number of plants showing infection. After the mold had grown for 46 days the culture of *Ps. tumefaciens* was no longer infective, indicating that the crown gall bacteria were not viable.

After 80 days in non-sterilized soil it was evident that there was a decrease in the number of plants showing infection. The galls produced were also smaller in size. At times during these periods of gall development, the temperature of the greenhouse was often relatively high, 25°-30° C., and the inoculated plants were exposed for several hours to direct sunlight. As shown by Riker (1925), at air temperatures of 28°-30° C., only small galls are formed, and above 30° C. no galls were produced from pure culture in-

oculations. Although only small galls were formed as a result of inoculations with infested soil, they were caused by the crown gall organism and not due to callus formation as a result of the inoculation wound. Isolations from such galls and reinoculation into tomato plants yielded typical galls as shown in plate 4, fig. B.

Similar trials were made with infested non-sterilized clay soil. Ten plants were inoculated and five plants pricked with a sterile needle as controls. The soil was infested Feb. 25, 1926, in the same manner with the strain of *Ps. tumefaciens* used in the previous trials. The results of these trials show that the crown gall bacteria were still viable and virulent on three out of ten tomato plants 122 days after soil infestation. The plants in this series were inoculated at the same time and remained under conditions identical with those in which infested compost was employed.

As shown in table V, only a few galls were produced after the inoculations of April 20. The results of the inoculations on April 20 and after were attributed to the high temperature in the greenhouse rather than to the dying out of the pathogen. In the infected plants the galls from the inoculations of April 20 were small in size as compared with those in former trials. The pathogen was recovered from these small galls and when inoculated into healthy tomatoes under shaded conditions (glass of greenhouses coated with green paint) produced typical large galls. No galls were produced on any of the control plants during either series of inoculations.

These results show that *Ps. tumefaciens* can remain in the soil for a considerable period of time without loss of virulence and, under favorable conditions, will produce typical galls on susceptible hosts.

## II. THE PATHOLOGICAL EFFECTS OF CROWN GALL ON APPLE AND PEACH TREES

The extent of the injury caused by overgrowths on the apple, whether due to crown gall or callus formation, has been a perplexing question for many years and there is little experimental evidence in support of the views of many investigators. Most of the early studies were observational in nature and made prior to the discovery of the causal organism of the disease. The literature of this period will not be reviewed here since it is fully summarized by Smith, Brown and Townsend (1911) and Hedgcock (1910).

Probably the first careful investigation of the pathologic effect of overgrowths on apple trees under nursery and orchard conditions was that of Hedgcock (1910). Although the cause of the overgrowths was not determined, he found: first, that relatively large galls encircling the one-year-old seedling root caused a perceptible stunting of the plant; second, that there was an average reduction of 3.7 inches in the height of the galled one-year-old trees and 5.1 inches for the galled two-year-old trees; third, that there was a reduction of 0.51 inches in trunk diameter for the galled trees after eight years in the orchard in comparison with normal trees of the same age.

Later, Swingle and Morris (1918) measured trunk circumference of eight varieties of apple trees after eight years in the orchard and found a reduction of 1.58 inches for the galled trees.

Greene and Melhus (1919) measured trunk diameter, twig length and twig weight on Jonathan and Wealthy apple trees after four seasons in the orchard. The reduction in trunk diameter for the galled Jonathan trees

was 11.3 percent, while for the Wealthy trees it was 21.7 percent, as compared with healthy trees of the same varieties. There was also a reduction of 25 percent in twig length and 37 percent in twig weight of the galled Wealthy trees. The galled Jonathan trees showed a reduction of 33.4 percent in twig length and 42 percent in twig weight.

Reddick and Stewart's (1924) observations on a small experimental orchard of budded apple and peach trees led to the conclusion that crown gall "is not a factor in the growth and development of either apple or peach trees." The fact that their observations on the apple were based upon a few trees of eight varieties, coupled with the variation in reaction of the individual to infection, would seem to render this conclusion inapplicable except under local conditions.

The question arises as to how the injurious effects of crown gall are produced.

That injury results from the abstraction of food materials and water by the rapidly developing gall tissue is stated by Smith, Brown and Townsend (1911), who also suggest the possibility of toxic substances, secreted by *Ps. tumefaciens*, or saprophytic forms within the gall, being absorbed by tissues remote from the gall. On the other hand, Woodworth (1892), Toumey (1900) and Swingle and Morris (1918) all suggest that the presence of a gall causes an obstruction to the upward sap flow thru the tree. If a gall at the union of root-grafted apple trees interferes with conduction, may not an imperfectly fitting graft have a similar effect? This question led to a consideration of the practices of root-grafting and of cutting back the yearling trees as to what might be their relation to the conduction of water and mineral nutrients through the tree.

This brief review points out that previous studies on the effect of overgrowths have been made on apple trees set in the orchard with varying degrees of infection. It cannot be denied that such data are extremely valuable, but it is also obvious that a long period of time would be required to accumulate a sufficient amount to estimate accurately the injury to the tree.

Such being the case, an attempt was made to devise some other method by means of which the probable injury caused by overgrowths and hairy root formations could be measured more accurately and rapidly. Early trials were made (Muncie and Berkhout 1925) in which a safranin solution was passed by means of a mercury manometer through galled and normal apple specimens. These showed that the passage of stain through the galled specimens was considerably retarded and also that the stain did not appear on the cut surface of the specimen above the overgrowth, showing interference to upward conduction. After a considerable amount of preliminary work, the rate of water flow was selected as the one function of the tree which might indicate its probable response under normal conditions in the field.

In this section are presented the results of studies on the rate of water flow in four varieties of apple from 1 to 13 years old and in four varieties of two-year-old peaches. Before presenting the data on water flow, it is deemed advisable to describe the trees used and the apparatus and methods employed in securing these measurements.

## MATERIALS AND METHODS

*Fluometer.*

A laboratory method was developed for the quantitative tests on water flow interference with upward conduction. The apparatus, designated a "fluometer," and the method employed, previously described by Melhus, Muncie and Ho (1924), consists essentially of a filter pump attached to a graduated burette into which water is pulled through the specimen and accurately measured. The period of suction in each case was five minutes.

In the measurements of rate of water flow, as given in tables VI and IX, air in the vessels was removed by a preliminary period of suction at a reduced pressure equivalent to 72.5 to 74 cm. of a mercury column for five minutes. This high pressure made it difficult always to prevent leakage around the connections, as well as to insure total removal of the air. Since a lower pressure obviously would minimize this error, a modification of the method was used in later tests.

Knowing from the work of Dixon (1914) that the transpiration current is equal to a velocity of flow created by a pressure head twice the perpendicular length of the specimen (15 centimeters), a negative pressure equivalent to 30 cm. of a mercury column was employed. The removal of the air was accomplished in much the same manner as that described by Farmer (1918), i. e., the specimens, after being cut under water, were placed in air free water and subjected to vacuum treatment. The removal of the air from the pieces was accomplished as follows:

The specimens in air free water were placed in a pressure cooker, the lid and safety valve of which were sealed with vaseline and paraffine. The pressure cooker was then connected with the fluometer by means of pressure tubing wired to the exhaust pet-cock, and the air exhausted. By this means the negative pressure, under which evacuation was accomplished, was registered on the mercury column of the apparatus. The duration of the vacuum treatment was three hours at a reduced pressure equivalent to 60 centimeters of a mercury column.

A series of consecutive trials was now made on the specimens so treated. To prevent clogging of the vessels, due perhaps to the diffusion of substances from the cut end of the specimen immersed in water, a fresh cut was made between each trial. While the readings were not constant for every specimen, many of them were, and a great majority checked within 0.4 c.c. in three consecutive trials. This figure was taken as the maximum variation in water flow allowable for any specimen in three consecutive trials.

*Specimens.*

The specimens employed in these tests were 15 centimeters in length. One, two or three specimens were taken from each tree. These will be referred to respectively as the union, scion and trunk pieces. The union piece included the union and equal lengths of root and stem. The scion piece, immediately above the union section, is so named because it includes the original scion of the graft and the offset in the stem caused by cutting back the yearling tree. The trunk piece was taken next above the scion piece. Text fig. 1 indicates the portion of the tree from which these specimens were taken.





Fig. 1. Diagram showing portion of tree from which specimens employed in tests were taken: (a) trunk piece; (b) scion piece; (c) union piece.

### Measurement.

The trees used in the water conduction tests were not of the same diameter, hence it was necessary, in order to make the results comparable, to establish a unit of measure. The greatest and least diameter of the upper cross section surface of the specimen were measured and the average of the two taken as the diameter of a circle. From this the area of the cross section surface was determined. The actual amount of water pulled through the specimen was then divided by this area, giving the flow through one square centimeter of surface. The result obtained per five minute period is designated as "the unit rate of flow." The rate of flow through the specimen irrespective of its diameter is designated as "the actual flow." The area of the cross section surface occupied by the pith, practically the same in all cases, was disregarded in the calculations.

### Terms.

Crown gall or gall as here employed refers to an excrescence or overgrowth on the root, union or trunk of nursery trees irrespective of the cause. From the results of cultural and histological studies it was evident that some of them were caused by *Ps. tumefaciens*, while others were due to excess callus at the union. Because of the treatment given the galled specimens in the measurement of water flow, no attempt was made to separate them on the basis of causation. The overgrowths, in some cases, were relatively soft and in others hard and woody, with a convoluted or smooth surface. Lateral roots sometimes arose from the galls, or, as in many cases, they were covered with fine fibrous roots. This latter condition is referred to in this paper as hairy root.

### Trees.

The galled and healthy trees of the varieties Jonathan, Wealthy and Ben Davis employed in the first series of fluometer tests were one-year cutbacks, selected from the nursery rows during the latter part of April by nursery employees. The apple trees employed in the second series of tests consisted of the same varieties as the above and were selected by the writer from the same rows, July 4, 1924. They were "heeled-in" out of doors until ready for study.

The two-year cutback trees employed in the later trials were obtained from two nurseries, one in Iowa and the other in Missouri. The galled Wealthy and Salome trees were selected from the discards at digging time by Dr. Melhus and the writer because of the large overgrowths at the union. The healthy trees of these varieties were number one trees selected by the nursery and stored in the nursery cellar until ready for fluometer tests.

The galled and healthy Jonathan trees were two years old, selected by the nursery employees and shipped to us from storage. Galled and healthy two-year-old peach trees of the varieties Elberta, J. H. Hale, Carman and Salway were secured from a Missouri nursery. The galled trees were badly infected; the galls in general half encircled the stem and in



many cases were twice the diameter of the stem. Galled trees typical of those employed in the trials are shown in plate I, A and B.

#### WATER CONDUCTION IN NORMAL YOUNG APPLE TREES

In studying the interference with water conduction due to crown gall, one of the first considerations was to measure the rate of water flow in young normal piece-root grafted apple trees as they occur in the nursery. This study raised the question as to the obstruction caused by the union of stock and scion and the "offset" caused by the practice of "cutting back" after the tree has made one season's growth.

Attention was first given to the extent of the obstruction caused by the union of the stock and scion. This was determined by comparing the rate of water flow through the union and trunk pieces of two-year "cutback" apple trees of the varieties Wealthy and Jonathan, using the fluometer as above described.

In these tests the specimens were prepared as follows: Trees "heeled-in" were brought into the greenhouse and cut into proper lengths as previously described. Before attaching the specimens to the apparatus, a fresh smooth cut was made on both ends of the piece. A preliminary period of suction was given each specimen until no bubbles of air arose into the burette, indicating that the vessels of the wood were exhausted of air as far as possible. In these trials and in the preliminary period of suction a negative pressure equivalent to 72.5 to 74 cm. of a mercury column was maintained, and the duration of the suction was five minutes. Three specimens were taken from each tree, namely, the union, scion and trunk pieces. The pieces were not cut from the tree until everything was ready for making the tests so as to avoid drying out of the wood. The results of these trials are presented in the histogram, fig. 2.

#### *Discussion of Data in Fig. 2.*

In an examination of the data in fig. 2 we find a wide variation in the unit flow values (0.8 c.c. to 5.2 c.c.) for trunk-piece specimens of the same variety. Such a variation would not appear so striking if it occurred in the rate of flow through the union or scion piece, but in the trunk pieces, without any obstruction, it emphasizes the extreme variability of trees of the same variety and grown under the same nursery conditions. When the fit of the graft, vigor of stock and position of the tree in the row are considered, the variation in the behavior of the trunk specimens loses its significance with regard to the results of these tests. It becomes obvious that in such a study a large number of trees must be employed to arrive at any definite conclusions.

The results of the trials on the Jonathan trees show a slight reduction amounting to 10.0 percent in the average unit flow through the union as compared with that through the trunk piece. While this difference in percentage is not high, if we consider the relative diameters of the two sets of specimens, the results become more significant. In the union pieces the average diameter is 1.81 cm. and the average unit flow is 2.16 c.c., while with an average diameter of only 1.44 cm. the unit flow through the trunk pieces is 2.40 c.c.

In the Wealthy trees a similar condition exists showing a reduction in unit flow through the union pieces amounting to 47.9 percent. With an average diameter of 1.92 cm. the unit flow is only 2.70 c.c. through the union

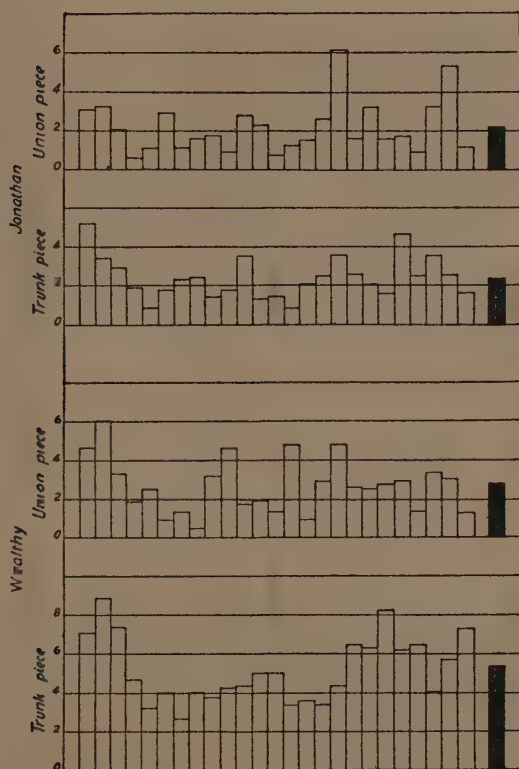


Fig. 2. Histogram showing the unit rate of water flow through the union and trunk pieces of normal two-year cutback Wealthy and Jonathan apple trees. The mean unit rate of water flow is shown by the solid column. Compare the data for the union pieces with those for the galled trees given in table VII.

piece, while through the trunk piece, with an average diameter of 1.54 cm., the unit flow is 5.18 c.c.

Supplementary tests were made to show the effect of the union and of cutting back upon water flow. For these additional tests, however, younger trees were studied. Healthy trees of each of the varieties Wealthy, Jonathan and Ben Davis, one and one-half year cutback trees, were employed. The trees were dug in April, 1924, and "heeled-in" for a period of about one week until ready for study. The results of these trials are given in the histogram, fig. 3.

#### *Discussion of Data in Fig. 3.*

Turning to the data on the Wealthy trees, we find that the average diameters of the union, scion and trunk pieces are 2.07 cm., 1.71 cm. and 1.52 cm., respectively. One would expect that the unit rate of flow through the piece would be proportional to the diameter. However, the data show the average unit flow for these specimens to be in the reverse order. The trunk piece with the smallest diameter had the highest unit flow, 7.55 c.c., while the union piece having the greatest diameter had a unit flow of only

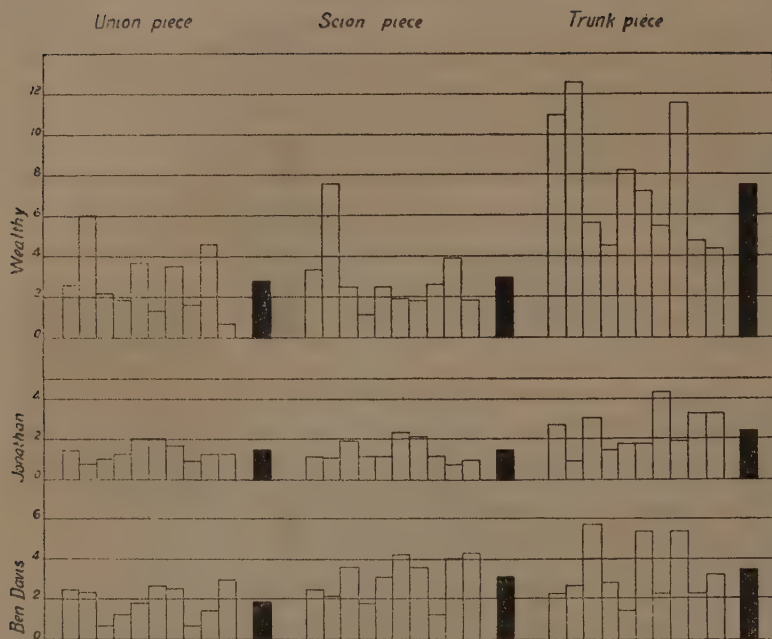


Fig. 3. Histogram showing unit rates of water flow through union, scion and trunk pieces of normal one and one-half year old cutback Wealthy, Jonathan and Ben Davis apple trees. The mean unit rates of water flow are shown by the solid columns. Compare the rates for the union pieces of these trees with those of the galled trees given in table VI.

2.81 c.e., the lowest of the three pieces. The unit flow through the scion piece, amounting to 2.91 c.e., was slightly greater than that through the union, but when one considers the greater diameter of the union piece, this difference appears significant. The average reduction in unit flow through the union and scion pieces, as compared with that through the trunk piece, was 62.8 and 62.5 percent, respectively.

In the Jonathan trees the average diameters for the union, scion and trunk pieces were 1.65 cm., 1.46 cm. and 1.26 cm., respectively. The unit flow through the union piece was 1.34 c.e., the scion piece 1.33 c.e., and the trunk piece 2.40 c.e. As in the Wealthy, we find a higher unit flow through the trunk piece, although the diameter was smaller, than through the union piece. The scion piece, with an average diameter of 0.46 cm. smaller than that of the union piece, had a unit flow of 0.01 c.e. less. This slight difference in unit flow, representing the average of 10 specimens, does not appear significant. In fact, considering the smaller diameter of these pieces, we might reasonably expect a greater difference between the two. The effect of the union and the offset due to cutting-back the tree is seen in the reduction in unit flow of 44.2 and 44.6 percent when compared with the unit flow through the trunk piece.

The data from the Ben Davis trees are similar to those from the trees of the other two varieties. While the average diameters of the union, scion

and trunk pieces were 1.80 cm., 1.46 cm. and 1.29 cm., respectively, the unit flow through the specimens was in reverse order to their diameters; the trunk piece 3.29 c.c., the scion piece 3.03 c.c. and the union piece 1.88 c.c. In this variety cutting back had caused a reduction in the unit rate of flow of 7.9 percent, but the decrease in unit flow through the union as compared with that through the trunk piece amounted to 42.9 percent.

The effect of the union in piece-root grafted apple trees was clearly shown by the reduction in rate of water flow in the varieties tested. As would be expected, there was a wide variation in the individuals, which is evidenced also in the averages for the different varieties and lots of the same varieties. However, if we take the average of 80 individuals of the three varieties studied, we find that the unit rate of flow through the union pieces was approximately 43 percent lower than that through the trunk pieces. The offset due to cutting back the yearling tree caused a reduction of

TABLE VI. WATER FLOW THROUGH UNION PIECES OF GALLED ONE AND ONE-HALF-YEAR CUTBACK APPLE TREES.

Galled Trees				Description of Gall			
Variety	Tree numbers	Diameter cm.	Unit flow c. c.	Height cm.	Length cm.	Percent girdling	Roots from gall and diam. in.
Wealthy	15*	1.6	2.9				
	16*	1.6	3.4				
	17*	1.6	2.8				
	18*	1.7	3.0				
	19*	1.7	2.7				
	21*	1.7	0.7				
	22*	1.7	1.9				
	23*	1.8	3.1				
	52	2.1	7.0	2.0	2.5	50	
	53	2.1	1.1	2.5	2.5	50	2- $\frac{1}{4}$
	54	2.2	0.7	2.0	1.2	25 <sup>1</sup>	
	55	2.1	2.9	2.0	2.5	50	1- $\frac{1}{4}$
	56	2.0	2.2	2.5	2.0	50 <sup>2</sup>	3- $\frac{1}{8}$
	57	2.4	0.6	Flat	4.0	50	
	58	2.2	1.5	2.5	2.0	.50	
	59	2.3	1.0	1.2	1.2	12.5	
	60	2.2	1.0	Flat	3.5	75	
Average		1.94	2.26				
Jonathan	24*	1.7	4.9				
	25*	1.7	1.6				
	26*	1.6	3.2				
	27*	1.7	2.1				
	28*	1.8	0.0				
	30	1.7	0.7	3.7	3.7	50	1- $\frac{1}{2}$
	31	1.7	0.3	3.7	1.2	50 <sup>2</sup>	
	32	1.9	0.7	2.0	1.2	75	
	33	2.3	0.0	1.2	1.2	25	
	34	2.1	1.1	5.0	2.5	75	4- $\frac{3}{8}$ :1- $\frac{1}{2}$
	35	2.2	1.1	1.2	2.0	25	
	36	2.1	0.0	3.7	1.2	50	2- $\frac{1}{4}$ :6- $\frac{1}{2}$
	37	2.0	0.7	2.0	1.2	20	1- $\frac{1}{2}$ :5- $\frac{1}{4}$
	38	2.0	0.9	3.5	1.3	50	5- $\frac{1}{4}$
	39	2.3	0.2	2.5	1.3	50	4- $\frac{1}{2}$
	40	2.3	1.0	3.7	3.0	66	3- $\frac{1}{4}$
	41	1.9	1.0	2.5	1.2	50	1- $\frac{1}{2}$
Average		1.94	1.10				
Ben Davis	42	2.0	1.4	2.0	2.0	50	1- $\frac{1}{4}$
	43	1.9	2.5	2.5	2.0	75	1- $\frac{1}{4}$ :5- $\frac{1}{8}$
	44	2.4	1.0	1.2	2.5	50	3- $\frac{1}{4}$ :3- $\frac{1}{2}$
	45	1.9	2.8	3.7	3.7	75	
	46	2.4	0.5	2.5	2.0	50	6- $\frac{1}{4}$
	47	2.1	1.0	2.5	1.2	25	3- $\frac{1}{4}$
	48	2.1	0.5	1.2	1.2	25	
	49	2.1	0.6	Flat	2.5	50	4- $\frac{1}{2}$
	50	2.2	1.0	2.5	1.2	25	3- $\frac{1}{4}$
	51	2.1	0.9	1.2	2.0	25	
Average		2.12	1.22				

\* Specimens used for sectioning.

<sup>1</sup> One  $\frac{1}{2}$  inch root opposite gall.

<sup>2</sup> Two  $\frac{1}{4}$  inch roots opposite gall.

<sup>3</sup> Gall on scion above union.

44.6 and 62.5 percent in the unit flow through Jonathan and Wealthy trees, respectively, but for the Ben Davis trees it was 7.9 percent. The interference due to cutting back was probably induced by the small amount of sapwood developed below the offset. In other words, the old scion did not grow as rapidly as the new shoot and only a narrow layer of sapwood served to supply the parts above it. Cross sections of the respective pieces supported this suggestion.

The significant point brought out in these results and that bearing directly upon the problem was the water flow interference in the union. Such interference as noted above, in an average of 80 trees of three varieties, amounted to 43 percent. In other words, the unit rate of flow through the union was only 57 percent of that through the trunk piece where there was no artificial obstruction.

Still another point deserving special emphasis in connection with this study of normal trees was the difference in unit rate of flow for the three varieties studied. While the extent of healing of the union of stock and scion probably accounted to some extent for the difference in unit flow through the union pieces, such could not be the case with the trunk pieces. In the case of the Wealthy trees, with an average diameter of trunk 0.1 cm. greater than that of the Jonathan, the water conducting capacity was doubled. This greater capacity for water flow through the trunk may be an important factor in determining the relative growth of the two varieties as well as their ability to withstand severe winter conditions.

#### EFFECT OF CROWN GALL ON WATER FLOW

Having studied the water flow in 80 normal apple trees of three varieties, Jonathan, Wealthy and Ben Davis, and having found the average rates of flow through the trunk, scion and union pieces to be 4.02 c.c., 2.41 c.c. and 2.42 c.c., respectively, consideration was given to galled trees, especially those having galls on the union. Two lots were used, differing slightly in age. The first lot consisted of one and one-half year cutbacks of the varieties Wealthy, Jonathan and Ben Davis, and the second lot of two year cutbacks of the varieties Wealthy and Jonathan. The results of the trials on water flow, using healthy trees of the same varieties as controls, are recorded in tables VI and VII and figs. 2 and 3.

#### *Discussion of Data in Table VI and Fig. 3.*

On examination of the data from the tests with the Wealthy trees in table VI we note a rather wide margin in rate of water flow through both the healthy and galled specimens. In the healthy trees the variation was from 0.7 c.c. to 6 c.c.; in the galled trees a slightly greater variation exists, namely, 0.7 c.c. to 7 c.c. A comparison of unit flow in the galled trees numbers 52 and 16 with that of the healthy trees numbers 73 and 76 showed in the case of the galled trees an average unit flow of 0.35 c.c. greater than in the healthy trees. It will also be noted that the highest unit flow of 7 c.c. for all trees of the variety was in the galled class. The description of the trees shows relatively large lateral roots rising from the gall. We also note that the gall half encircles the union. These two trees, however, are exceptions to the general rule in this particular lot, which is evident from the averages for the healthy and galled lots as a whole. In this lot the average diameter and cross section area of the union piece of the healthy trees is about 0.1 cm. and 0.4 cm. greater than the galled trees. With this small



difference in the area exposed to suction, the unit flow of the healthy trees was 2.82 c.c., while for the galled trees it was 2.26 c.c. Thus, we find that the gall at the union reduced the rate of water flow 19.8 percent.

In the Jonathan trees there is little difference in actual rate of flow in the galled and the healthy specimens. The average diameter of the former is 1.94 cm., while that of the latter is 1.65 cm. However, with a larger diameter, the unit rate of flow through the galled union pieces is 1.10 c.c., as compared with 1.32 c.c. for the healthy pieces, a reduction of 16.7 percent in the galled trees. There is little individual variation in flow in the Jonathan trees as compared with the Wealthy. While there are two cases in which the actual and unit flow of the galled trees is greater than

TABLE VII. WATER FLOW THROUGH UNION PIECES OF GALLED TWO-YEAR CUTBACK APPLE TREES.

Variety	Galled trees			Description of gall		
	Tree number	Diameter cm.	Unit flow c. c.	Height cm.	Length cm.	Percent girdling
Wealthy	168	1.9	3.5	1.2	1.2	33
	169	1.9	3.4	Flat	3.7	50
	170	2.2	1.9	Flat	3.5	100
	171	1.8	2.0	2.5	2.0	50
	172	1.8	3.2	1.2	0.5	25
	173	2.3	0.8	2.5*	2.5*	50*
	174	1.9	3.3	1.2	1.2	25
	175	1.9	3.3	1.2	2.0	25*
	176	1.9	4.0	Flat	3.0	100 <sup>1</sup>
	177	1.4	1.7	1.2	1.2	25
	178	1.8	1.9	Flat	2.5	75
	179	2.1	3.8	2.0	1.3	33*
	180	2.0	1.3	0.6	0.2	16
	181	2.3	1.0	Flat	2.5	100
	182	1.9	1.4	Swollen union Swollen union		
	183	1.8	1.6			
	184	1.9	2.3	0.6	0.7	16
	185	2.2	2.0	2.5	2.0	50*
	186	2.1	1.4	0.5	0.7	16
	187	1.6	4.0	Flat	2.5	50
	188	1.9	1.7	1.2	1.3	25*
	189	2.2	1.2	1.2	2.5	33*
	190	1.6	0.8	1.2	1.2	25
	191	2.4	1.2	2.5	2.5	50*
	192	2.0	7.5	0.6	2.0	100
Average		1.95	2.36			
Jonathan	118	1.8	1.2	1.2	2.0	50*
	119	1.6	0.8	1.2	0.5	25*
	120	1.2	7.1	1.3	2.0	50
	121	1.6	1.2	1.3	1.2	25
	122	1.7	1.6	0.6	0.7	16
	123	1.3	1.3	2.0	1.2	50
	124	2.0	2.9	0.5	0.5	16
	125	1.7	3.6	2.0	2.0	50
	126	1.5	2.0	1.2	2.0	50
	127	1.7	1.5	2.0	2.0	33
	128	1.9	0.6	0.5	0.7	16
	129	1.8	0.2	Flat	2.5	100
	130	1.6	1.9	1.2	1.5	25*
	131	1.3	1.1	2.0	2.0	50
	132	1.6	1.2	1.2	1.2	16
	133	1.3	1.3	0.5	0.5	12
	134	1.8	1.1	2.5	2.5	25
	135	1.6	0.6	Flat	3.7	100
	136	2.0	0.6	2.5	2.0	100
	137	1.6	2.7	1.2	0.6	12
	138	1.8	2.2	1.2	2.0	25
	139	1.2	0.5	2.5	2.5	50
	140	1.2	0.6	Flat	3.5	50
	141	1.3	0.6	2.0	2.0	50
	142	1.2	2.7	2.5	2.5	50*
Average		1.59	1.64			

\* Gall on trunk above union.

<sup>1</sup> One  $\frac{1}{8}$  inch and three  $\frac{1}{4}$  inch roots from gall.

<sup>2</sup> One  $\frac{1}{8}$  inch and six  $\frac{1}{8}$  inch roots from gall.

that of the healthy, the average for the entire lot is still less than that for the healthy trees.

In the Ben Davis trees, none of the galled ones shows a higher unit flow than the healthy trees. The variations in flow in the galled specimens are from 0.5 c.c. to 2.8 c.c. and for the healthy, from 0.7 c.c. to 3.0 c.c. The average diameter of the galled union pieces is slightly larger than that of the healthy. The average unit flow for the two lots shows that with the average diameter, 14 percent greater, the flow in the galled trees is 35.5 percent less.

Although the reduction in unit rate of flow in the galled Wealthy and Jonathan trees is less than that in the Ben Davis, taken as a whole, the results show that the gall on the union materially reduces the flow of water through the tree.

While the results obtained from the previous trials show that galls at the union of root grafted apple trees caused interference with the upward flow of water, a second series of trees was tested by the same method to obtain a better indication of the situation at the time the trees were ready for setting in the orchard. In these trials two-year cutback trees of the varieties Wealthy and Jonathan were used. This lot consisted of 25 galled and an equal number of healthy trees of each of the above varieties. Unless otherwise noted in the table, the galls occurred on the union of the tree. The results of these tests are given in table VII and fig. 2.

*Discussion of Data in Table VII and Fig. 2.*

For the galled Wealthy trees we find a greater average diameter (1.95 cm.) than that (1.88 cm.) for the healthy trees. There was only a slightly greater average actual flow, 7.75 c.c., in the healthy trees, as compared with 7.06 c.c. in the galled trees. However, when placed on a comparable basis we find the average unit flow of 2.7 c.c. for the healthy trees to be 12.6 percent greater than that of the galled trees, which amounts to 2.36 c.c. In the case of one galled tree the actual flow (23.6 c.c.) as well as the unit flow (7.5 c.c.) is higher than that of any of the healthy trees. In this case we note that the gall was flattened and the tree had an abundance of comparatively large roots. The effect of such a swelling at the union is so variable that one cannot predict its result upon water conduction through the union.

In the Jonathan trees we find a reverse condition from that occurring in the Wealthy trees. Here the galled trees are 0.21 cm. smaller in average diameter with an actual flow of 3.23 c.c. and unit flow of 1.64 c.c., as compared with 5.62 c.c. and 1.95 c.c. for the healthy trees. It is difficult to make a comparison of behavior between galled and healthy trees since it is possible that the development of conducting vessels in the pieces of smaller diameter is less than that in pieces of larger diameter. If this were true, we could not attribute the lower rate of flow to the effect of the gall. On the other hand, it is possible that this retardation in trunk growth might be due to the presence of the gall since these trees had the same chance for growth in the nursery row as the galled trees. Be that as it may, there was a reduction in unit flow of 12.4 percent in the galled trees as compared with that of the healthy trees. The results from these tests confirm those presented in table VIII and show that a gall at the union of two-year cutback Wealthy and Jonathan trees reduced the unit flow 12.6 and 12.4 percent, respectively.

Further measurements were made on two-year cutback Wealthy trees, using a modification of the previous method. This consisted essentially in cutting the specimens from the tree in such a way that the minimum amount of air would enter the vessels, allowing a more thorough vacuum treatment, and the employment of a lower negative pressure in suction. The results of the trials on healthy and galled trees is presented in table VIII and fig. 4.

*Discussion of Data in Table VIII and Fig. 4.*

The galled trees used in these tests showed an average diameter of 2.21 cm., while that of the healthy trees was 2.54 cm., an increase of 0.33 cm. In the galled trees the average actual flow was 14.8 c.c. and 47.94 c.c. in the healthy trees; approximately 3.4 times as great. The average unit flow of the galled trees was 2.9 c.c. and 9.58 c.c. in the healthy trees, or 3.3 times that of the galled trees. We note a wide variation in the unit flow of the galled trees, which appeared to be associated with the development of lateral roots from the gall. Thus in trees 574, 575, 578, 579, 581, 599, 604 and 605, where there were several relatively large roots from the gall, the actual and unit flows were correspondingly greater. An exception to this condition, however, was seen in tree number 577, in which, with no lateral roots from the gall, the actual flow was 34.4 c.c. and the unit flow was 8.3 c.c. This may be attributed to the lack of penetration of the gall causing little distortion of the conduction vessels. On the other hand, there were many galled trees with several lateral roots from the gall which showed a low actual and unit flow. Such cases are seen in trees numbers 618, 619, 620, 622, 623, 627, 628, 629, 630 and 631. This low actual and

unit flow may be attributed to the effect of the gall overbalancing the effect of the lateral roots in increasing the capacity for conduction. In other words, the presence or absence of large lateral roots may not indicate a high or low rate of water flow. The amount of vascular distortion or discontinuity is to a large extent the determining factor.

The rate of water conduction was not always dependent upon the degree of girdling of the union by the gall; a higher rate of flow sometimes occurred in trees completely girdled by a gall than in those only partially girdled.

As in the previous lots of Wealthy trees studied, the gall at the union presented

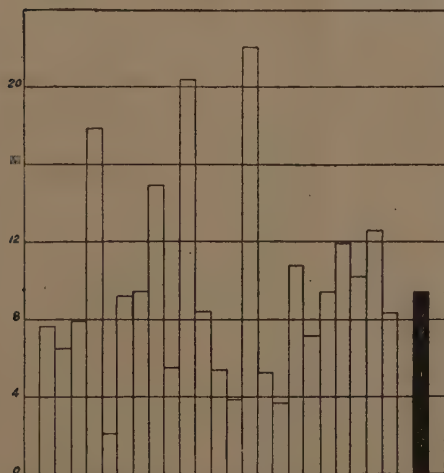


Fig. 4. Histogram showing unit rate of water flow through the union pieces of normal two-year cutback Wealthy apple trees. The mean unit rate of water flow is shown by the solid column. Compare with the data for the galled trees given in table VIII.

a decided interference with water conduction, causing a reduction in the unit rate of water flow amounting to 69.73 percent.

It seemed desirable to learn whether the effect of a gall on the union of other rapidly growing varieties was similar to that on the Wealthy. Trees of the variety Salome were selected for making this comparison be-

TABLE VIII. WATER FLOW THROUGH UNION PIECES OF GALLED TWO-YEAR CUTBACK WEALTHY APPLE TREES.

Galled trees			Description of gall			
Tree number	Diameter cm.	Unit flow c. c.	Height cm.	Length cm.	Percent girdling	Roots from gall and diam. inches
574	2.9	2.0	1.5	5.0	40	2- $\frac{1}{2}$ :1- $\frac{1}{4}$
575	2.5	6.7	1.4	4.0	50	0
576	2.5	4.6	1.7	3.5	50	2- $\frac{1}{4}$
577	2.3	8.5	1.5	4.0	60	3- $\frac{1}{4}$
578	2.5	24.8	2.5	6.0	40	4- $\frac{1}{4}$
579	2.7	24.8	1.2	3.0	35	2- $\frac{1}{4}$ :2- $\frac{1}{2}$
580	2.8	6.3	2.5	5.0	50	2- $\frac{1}{4}$
581	2.8	7.0	1.5	3.5	50	2- $\frac{1}{4}$
582	3.1	5.7	2.0	3.0	50	0
583	2.2	1.3	Flat	0.0	50	3- $\frac{1}{4}$
584	2.9	1.7	2.0	4.5	50	1- $\frac{1}{2}$
585	2.5	3.1	2.0	5.5	50	2- $\frac{1}{4}$
586	2.4	0.6	2.5	4.0	75	3- $\frac{1}{4}$ :1- $\frac{1}{2}$
587	2.8	0.9	2.0	5.0	75	1- $\frac{1}{4}$
588	2.7	2.8	1.5	4.5	25	2- $\frac{1}{4}$
589	2.0	1.4	2.5	3.5	30	2- $\frac{1}{4}$
590	2.7	0.4	1.5	6.0	30	2- $\frac{1}{4}$
591	1.9	0.4	1.5	4.0	50	3- $\frac{1}{4}$
592	2.1	0.6	2.5	4.0	60	0
593	2.9	0.9	0.5	3.5	50	0
594	2.8	1.5	1.5	3.2	75	0
595	1.8	0.2	0.5	4.5	33	0
596	2.7	2.4	1.0	3.0	50	0
			1.0	3.0	50	0
			0.7	2.5	33	0
597	1.8	0.5	2.5	4.0	100	1- $\frac{3}{4}$ :1- $\frac{1}{4}$
598	2.4	2.9	2.0	4.0	66	1- $\frac{3}{4}$ :1- $\frac{1}{4}$
599	2.2	8.9	3.0	3.5	33	3- $\frac{1}{4}$
600	2.4	6.6	3.6	5.0	50	2- $\frac{1}{4}$
601	2.7	6.2	1.5	3.0	50	2- $\frac{1}{4}$
602	2.6	5.1	2.0	4.0	33	3- $\frac{1}{4}$
603	1.6	4.8	1.5	4.0	50	2- $\frac{1}{4}$
604	2.4	8.6	2.0	4.0	50	3- $\frac{1}{4}$ :3- $\frac{1}{2}$
605	2.1	8.5	1.0	2.0	25	5- $\frac{1}{4}$
			1.2	4.0	50	0
			0.5	3.0	33	0
606	1.9	1.6	1.0	4.0	50	0
607	2.3	1.2	1.5	4.0	100	2- $\frac{1}{2}$ :1- $\frac{1}{4}$
608	1.4	1.3	1.3	4.0	50	2- $\frac{1}{4}$
609	2.0	0.3	2.0	5.0	100	0
610	2.8	1.9	0.5	2.0	25	0
			1.5	4.0	25	0
611	2.4	1.4	2.8	4.5	50	4- $\frac{1}{4}$
612	2.4	2.1	1.8	4.5	100	0
613	2.6	2.1	2.0	4.0	33	2- $\frac{1}{4}$ :4- $\frac{1}{4}$
614	2.0	1.1	2.0	3.0	50	1- $\frac{1}{4}$ :4- $\frac{1}{4}$
615	2.6	0.3	2.3	5.0	100	3- $\frac{1}{4}$
616	2.6	1.8	2.0	6.0	50	1- $\frac{1}{4}$
617	2.6	0.4	2.3	3.2	33	0
618	2.7	0.8	1.5	4.5	20	1- $\frac{1}{4}$
619	2.4	0.4	2.5	4.5	50	1- $\frac{1}{4}$ :1- $\frac{1}{4}$
620	2.3	0.1	2.0	4.5	50	1- $\frac{1}{4}$
621	2.2	4.3	1.2	3.0	25	0
622	2.2	0.3	1.0	6.0	100	1- $\frac{1}{4}$
623	2.6	0.5	1.0	3.5	100	1- $\frac{1}{4}$
624	2.6	0.1	1.6	4.5	50	0
625	2.3	0.7	1.7	3.5	50	0
626	1.9	0.8	1.1	4.0	50	0
627	2.1	0.2	1.5	4.0	33	3- $\frac{1}{4}$ :1- $\frac{1}{4}$
628	2.1	1.0	2.3	4.5	50	4- $\frac{1}{4}$
629	2.5	0.7	2.5	4.5	50	3- $\frac{1}{4}$
630	2.6	0.1	1.0	4.3	75	1- $\frac{1}{4}$ :3- $\frac{1}{4}$
631	1.7	0.4	2.0	4.6	75	10- $\frac{1}{4}$
2.21		2.90				

\* Galls on trunk above union.

cause a high percentage of them was affected in the field with large galls at the union and they were available for study.

The results of the trials on healthy and galled trees of this variety are presented in table IX and fig. 5.

*Discussion of Data in Table IX and Fig. 5*

From these data we find that the average diameters of galled and healthy Salome trees differed very little; the former being 2.27 cm. and the latter 2.08 cm. There was little difference between the average actual rate of flow of the healthy trees (24.4 c.c.) and that of the galled trees (21.3 c.c.). However, when we consider the unit flow for galled and healthy trees, we find they were 4.52 c.c. and 5.77 c.c., respectively, or a reduction in the galled trees of 21.7 percent. The effect of lateral roots from the gall on increased water flow as exemplified in trees number 455, 471, 480, 484, 494 and 495 was noticeable. The effect of lateral roots from

TABLE IX. WATER FLOW THROUGH UNION PIECES OF GALLED TWO-YEAR CUTBACK SALOME APPLE TREES.

Tree number	Galled Trees		Description of gall			
	Diameter cm.	Unit flow c. c.	Height cm.	Length cm.	Percent girdling	Roots from gall and diam. inches
452	1.4	1.4		callus		
453	2.5	5.8	1.5	4.0	75	1- $\frac{1}{8}$
455	1.9	8.1	[ 1.0	2.0	25	1- $\frac{1}{8}$
			1.0 a	2.0	25	1- $\frac{1}{8}$
			0.5	3.0	25	1- $\frac{1}{8}$
456	2.4	3.0		callus		
457	1.7	1.6				
458	1.5	1.8	0.8	3.5	25	3- $\frac{1}{8}$
467	2.1	5.6	0.5	1.5	16	2- $\frac{1}{8}$ *
462	1.9	5.6		callus		
464	2.4	12.0	1.5	3.0	25	2- $\frac{1}{8}$ :2- $\frac{1}{8}$ *
465	2.8	4.9	0.3	1.5	20	1- $\frac{1}{8}$ *
466	3.0	1.9	2.0	3.5	50	10- $\frac{1}{8}$ :3- $\frac{1}{8}$
467	1.6	1.3	2.0	3.0	100	
468	2.0	5.9		callus		
469	2.5	5.0	[ 1.0	2.0	12	1- $\frac{1}{8}$
			1.0	2.0	12	
470	2.7	6.9		callus		
471	1.5	6.3	0.7	3.5	50	1- $\frac{1}{8}$ :1- $\frac{1}{8}$
472	1.6	1.0	1.0	2.0	50	1- $\frac{1}{8}$ :3- $\frac{1}{8}$
473	2.3	3.9	0.6 b	1.5	25	
474	2.1	5.8	1.0 b	3.0	25	3- $\frac{1}{8}$
476	2.1	1.8	0.2	1.0	25	4- $\frac{1}{8}$ *
477	1.4	5.7		callus		
478	1.5	6.4	1.0	3.0	50	2- $\frac{1}{8}$
479	2.2	7.3	0.5	2.0	25	4- $\frac{1}{8}$ :1- $\frac{1}{8}$
480	2.5	8.8	1.5	3.0	50	5- $\frac{1}{8}$
481	1.8	5.8	2.0	4.0	33	2- $\frac{1}{8}$ :1- $\frac{1}{8}$
482	2.4	5.2	1.5 a	3.3	50	
483	1.7	2.5	1.0 b	2.5	50	
484	2.5	7.0	0.9 a	3.0	20	2- $\frac{1}{8}$
485	2.1	9.7	0.5	2.5	75	4- $\frac{1}{8}$ *
486	2.5	3.1	1.0	callus		
487	1.6	24.0	1.0	0.5	12	1- $\frac{1}{8}$ *
490	2.5	3.6	0.5	4.0	50	1- $\frac{1}{8}$
493	2.8	5.7		callus		
494	2.4	8.3	1.0	3.0	25	1- $\frac{1}{8}$
495	2.4	8.68	0.5	1.0	12	3- $\frac{1}{8}$
496	2.1	7.31	1.0	3.3	25	1- $\frac{1}{8}$ :4- $\frac{1}{8}$
498	1.7	3.70		callus		
501	2.0	5.32	1.0	2.0	25	1- $\frac{1}{8}$ :1- $\frac{1}{8}$ *
502	2.0	6.39		callus		
503	1.7	1.81	0.5 b	2.0	33	1- $\frac{1}{8}$ :1- $\frac{1}{8}$
	2.08	4.52				

a Gall on scion.

b Gall on stock.

\* Root from below gall.

\* Root from union opposite gall.

\* Root from above gall.



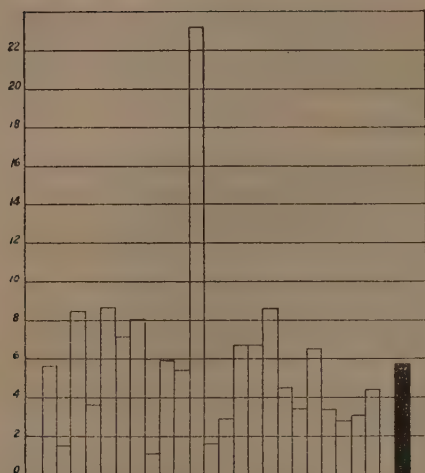


Fig. 5. Histogram showing unit rate of water flow through union pieces of normal two-year cutback Salome apple trees. The mean unit rate of flow is shown by the solid column. Compare with the data for the galled trees given in table IX.

the union opposite the gall was shown in tree number 464, which showed the highest unit flow of any of the galled trees.

Only one of the galls completely encircled the union in this variety, hence no comparison can be made as to the effect of girdling since the variation in flow through trees only partially girdled is too great. The size of gall appeared to have little correlation with reduction in unit flow in this lot of trees. The interference due to the gall on the union of Salome trees was not as striking as that of the Wealthy, and the unit rate of water flow was reduced about 22 percent as compared with the healthy trees (fig. 5).

Having made measurements upon two-year cutback trees of different ages and of four varieties, further tests were carried out employing healthy and galled two-year-old Jonathan trees. The galled trees were taken from a lot of discards labelled "knotted," but which on further examination showed only about one-third of them to be galled, the remainder having swollen unions or a poor root system. After making the measurements on water flow, the specimens were split through the center of the scion lip and classified as being galled or showing a swollen union.

The results of these trials on galled and healthy trees are given in table X and fig. 6.

#### *Discussion of Data in Table X and Fig. 6.*

As shown in the table, the galled trees had an average diameter slightly greater than the healthy. With a greater diameter and area of surface exposed to suction, the unit rate of flow for the galled trees was 47.2 percent lower than that of the healthy.

Only a few of the galled trees of this variety had lateral roots arising from the gall. Tree number 558 with four roots from the gall one-eighth inch in diameter showed a unit rate of flow of 12.2 c.e., while tree number 556 with roots of the same size and number had a unit flow of only 7.6 c.e. Again, in tree number 560 with no roots from the gall, the unit rate of flow was 21.1 c.e. This flow value was nearly as large as many of those found for the healthy trees.

A number of the healthy trees showed a swollen union due to callus formation. It is interesting to note the unit rates of water conduction

in these trees, which, with a few exceptions, are lower than those of the trees with smooth unions. In these exceptional cases, trees number 562 and 565, the unit rate of water flow was quite as high as that of a majority of the normal trees. Even though such a swollen union does not always mean that the water conducting capacity is low, it appears quite indicative of interference to a greater or less degree.

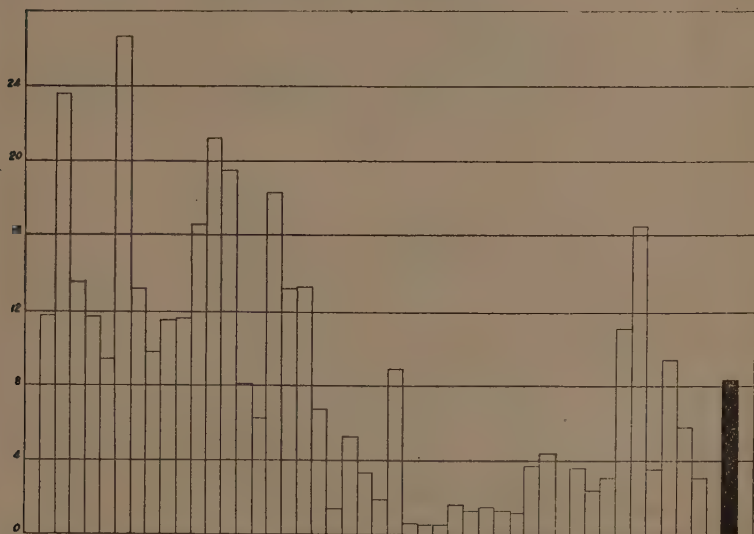


Fig. 6. Histogram showing unit rate of water flow through union pieces of normal two-year-old Jonathan apple trees. The mean unit rate of flow is shown by the solid column. Compare with data for galled trees given in table X.

TABLE X. WATER FLOW THROUGH UNION PIECES OF GALLED TWO-YEAR JONATHAN APPLE TREES.

Galled trees			Description of gall			
Tree number	Diameter cm.	Unit flow c. c.	Height cm.	Length cm.	Percent girdling	Roots from gall and diam. in.
530	2.0	1.4	1.0	1.5	50	1- $\frac{1}{8}$
531	1.6	1.2	0.5 <sup>2</sup>	1.0	50	1- $\frac{1}{8}$
532	1.6	3.8	1.0	2.0	25	0
536	1.6	1.4	0.5	1.0	66	0
538	1.6	3.4	0.5 <sup>2</sup>	2.0	25	0
549	1.6	1.3	0.5	2.0	75	0
554	2.0	4.4	0.5	2.0	25	3- $\frac{3}{8}$
556	1.9	7.6	1.7	3.0	33	4- $\frac{1}{2}$
558	2.0	12.2	0.5 <sup>1</sup>	2.0	25	4- $\frac{1}{2}$
559	1.9	2.0	1.0 <sup>1</sup>	2.0	50	0
560	2.0	21.1	0.5	1.5	33	0
561	1.8	1.7	0.7	3.5	100	0
563	1.4	2.2	1.5 <sup>2</sup>	2.0	100	3- $\frac{1}{8}$
566	1.8	0.2	1.1 <sup>1</sup>	2.0	50	0
			0.5	2.0	16	
567	2.1	1.9	1.2	4.0	50	1- $\frac{1}{8}$
570	1.2	4.3	1.0	2.5	100	1- $\frac{1}{8}$
	1.75	4.38				

<sup>1</sup> Gall on scion.

<sup>2</sup> Gall on stock.

## TRIALS ON BUDDED PEACH TREES

Thus far all of the studies on interference with water flow have been made on piece-root grafted apple trees. The obstruction due to the gall on the peach may be less because of the absence of the root graft or it may be greater on account of the more rapid growth of the excrescence in the softer tissue. Isolations were made from five typical galled specimens before the fluometer trials, and *Ps. tumefaciens* was recovered in every case.

Galled and healthy peach trees were selected from the varieties Elberta, J. H. Hale, Carman and Salway. The specimens on which the measurements of water flow were made were taken below the point of budding so as to include equal portions of the root and trunk. Representative specimens are shown in plate I, fig. B. The results of the trials on these lots of trees are presented in the histogram, fig. 7.

*Discussion of Data in Fig. 7.*

There is a striking reduction in actual and unit flow through galled trees as compared with that of the healthy trees. With an average diameter of 1.76 cm., the average actual and unit flow for the galled trees was 1.23 c.c. and 0.55 c.c. The healthy trees, with an average diameter of 1.52 cm., showed an actual and unit flow of 6.9 c.c. and 3.7 c.c., respectively. Thus, in spite of a larger diameter in the galled trees, the unit rate of water flow is reduced approximately 83 percent.

The healthy Elberta trees are comparable to the healthy Hale trees in actual and unit flow and would appear to fall in the same class on the basis of conduction. In this variety the average actual flow was 6.05 c.c. and the unit flow was 3.77 c.c. The galled trees having a larger average diameter showed a low actual and unit flow, 1.15 c.c. and 0.58 c.c. as compared with that of the healthy trees. In the galled trees the reduction in unit flow was 84.6 percent.

In the healthy Carman trees the conduction was quite comparable to that of the healthy Salway trees. Here we find an average unit flow of 5.22 c.c. and an actual unit flow of 9.49 c.c. The trees of this variety, however, had a larger diameter than the Salway trees, but the average unit flow was 0.76 c.c. less. The galled Carman trees, while showing higher actual and unit flow than those of either Hale, Salway or Elberta trees, also showed a reduction of 75.1 percent in unit flow when compared with the healthy trees of this variety.

A similar condition exists in the Salway trees having a larger average diameter in the galled than in the healthy trees. Comparable to the 0.43 c.c. and 0.15 c.c. of actual and unit flow in the galled trees, we have 9.31 c.c. and 5.98 c.c. for the healthy trees. The reduction in unit flow for the galled trees was 73.5 percent.

Interference with water conduction in the peach trees was due entirely to the presence of the gall, since the specimens were taken below the point of budding. The galls on the peach were unlike a majority of those on the apple in this one important respect, namely, that they penetrated deeper into the tissue of the stem. There was also evidence of decay of the tissues of the stem around the gall, which would account for the serious injury indicated by the fluometer tests.

From the data on conduction in the healthy trees we find that the varieties tested can be placed in two classes, one including the Carman and

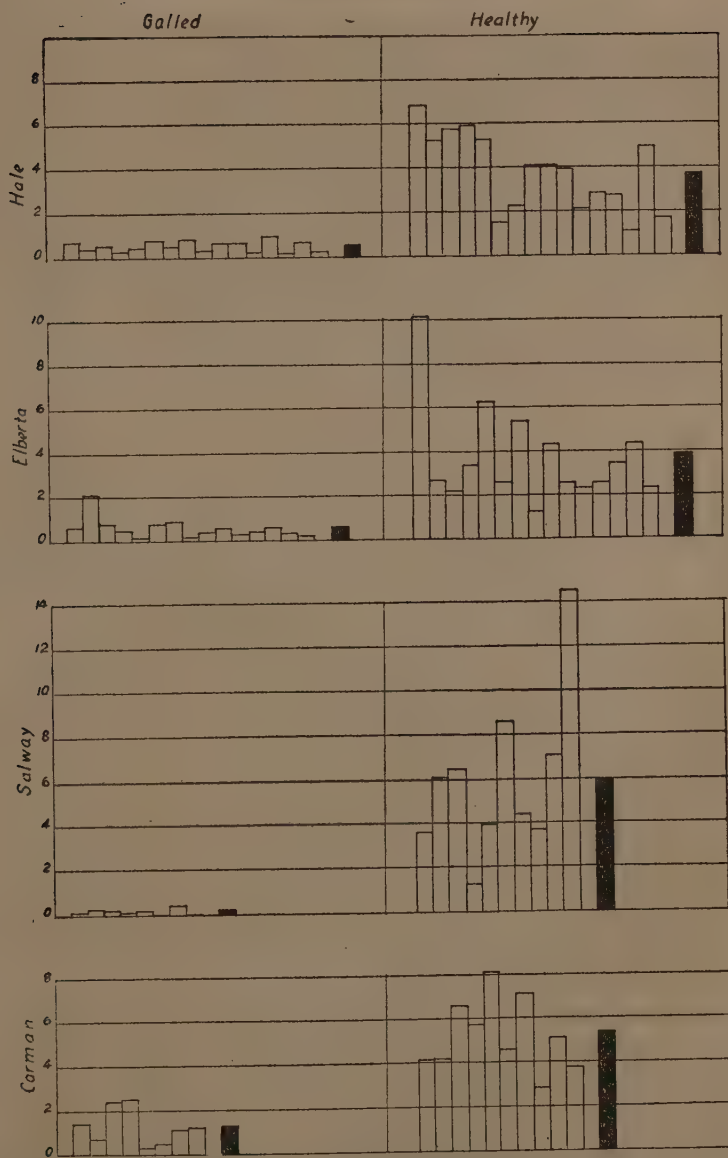


Fig. 7. Histogram showing unit rate of water flow through galled and healthy peach trees of the varieties, Hale, Elberta, Salway and Carman. The mean unit rate of flow is shown by the solid columns.

Salway with high average unit flow (5.6 c.c.), and the other including Hale and Elberta with relatively low unit flow (3.75 c.c.). In this connection it is interesting to note that the Salway and Carman peaches, under Iowa conditions at least, are regarded as more hardy than the Elberta and Hale. In the peach, then, we find the ability to withstand severe winter conditions correlated with high water conduction, thus corroborating the fact brought out in the Wealthy apple trees as compared with the Jonathan and Ben Davis varieties. From the results of water flow measurements on budded peach trees it is quite obvious that the presence of a gall offers a serious obstruction to conduction. This interference, expressed as a reduction of the unit flow through normal trees, amounted to 82.4 percent, an average for the four varieties. These measurements, made on specimens having no artificial obstruction to water flow, showed that the interference was caused entirely by the gall. In comparison with the average reduction due to the gall in piece-root grafted apples, it is evident that the peach is more seriously affected by crown gall.

#### RELATION OF TYPE OF GALL TO WATER FLOW INTERFERENCE

During the course of the studies on water flow through the galled apple trees, the question arose as to whether or not the interference with conduction could be correlated with the type of gall. The size of the gall seemed to offer the most obvious basis for such a study. In general observations, the size of a gall was measured by its most striking characteristic, namely, the extent to which it grew outward from the part of the tree to which it was attached. Less attention was paid to the relative proportion of the circumference of the trunk or root involved by the gall.

After measuring the rate of flow in the galled Wealthy, Salome and Jonathan trees as given in tables VIII, IX and X, the specimens were classified according to gross appearances, using size of the gall as a basis, and little regard was given to the extent of girdling. However, it was found that, because of their similarity in respect to projection from the union, this alone could not be accepted as a criterion of interference with water conduction.

After a more thorough examination of the specimens, they appeared to fit readily into groups based upon the extent to which the union was involved by the gall. Five classes were therefore made which included practically all the trees and consisted of specimens in which the gall involved 25, 33, 50, 75 and 100 percent, respectively, of the circumference of the union. The average unit rate of water flow, diameter of the specimens above the union, length of the gall and extent to which it projected from the union are summarized in table XI.

TABLE XI. RELATION OF SIZE OF GALL TO WATER FLOW INTERFERENCE.

	Number speci- mens	Average diameter specimen	Average unit flow	Percentage of union involved	Projection of gall from union	Length of gall
Class I .....	10	2.1 cm.	5.55 c. c.	25	0.80 cm.	2.48 cm.
Class II .....	9	2.4 cm.	4.60 c. c.	33	1.72 cm.	3.96 cm.
Class III .....	30	2.3 cm.	2.92 c. c.	50	0.88 cm.	1.98 cm.
Class IV .....	6	2.3 cm.	0.80 c. c.	75	1.58 cm.	3.85 cm.
Class V .....	9	2.0 cm.	1.25 c. c.	100	1.69 cm.	1.31 cm.



*Summary of Table XI.*

In examining the data in table XI, we first note the relative proportion of trees included in the various classes. Class number 3, in which 50 percent of the circumference of the union was gall tissue, included about 47 percent of the specimens studied. Classes one and two, in which 25 and 33 percent of the union were involved, contained about 30 percent, while classes four and five included the remaining 23 percent of the specimens. Turning now to the average rate of flow, it is interesting to note the rapid decrease in conduction as the percentage of gall tissue (extent to which the union was involved) increased. In class one and two, with the gall 25 and 33 percent encircling the union, the unit flow was 5.55 c.c. and 4.60 c.c., respectively, while in class three, with 50 percent of the union involved, the unit flow was only 2.92 c.c. Likewise, in classes four and five, with 75 and 100 percent of the union involved, the unit flow had been reduced to 0.80 c.c. and 1.25 c.c. The rate of flow in the trees of class five, with the entire union involved, was greater than in class four, where the gall encircled three-fourths of the union. This discrepancy was probably due to the small number of specimens in the latter class and their individual variations in conduction.

When we consider the average length of the gall and extent to which it projected from the union, we find that neither of these characters was correlated with the interference with water flow through the galled union. It is seen, however, that when the actual amount of galled tissue was considered, depending upon the length and extent of projection of the gall and the percentage of the circumference of the union involved, the size of the gall was quite closely correlated with interference with water flow.

## RELATION OF HEALING OF THE UNION TO WATER FLOW AND GALL FORMATION

From the previous measurements it was shown that the union of a piece-root grafted apple with or without a gall interfered with the water flow through the tree. Further studies, to determine the cause of this obstruction, were made on specimens of the Wealthy, Jonathan and Salome trees given in tables VIII, IX and X. The specimens were sawed longitudinally through the union, making it possible to observe the extent of healing of the union of stock and scion, the amount of callus tissue and the presence or absence of continuity of the water conducting vessels. The specimens were arranged into three classes. Class number one consisted of trees in which there was complete healing of the union; class number two, those in which the tip of the scion lip had failed to unite with the stock; class number three, those in which the tip of the stock lip had failed to unite with the scion. The numbers of healthy and galled trees falling within these classes and the average unit rate of water flow through the union are given in table XII.

TABLE XII. EFFECT OF HEALING OF UNION ON RATE OF FLOW.

Class	Healthy trees		Galled trees	
	Number trees	Average unit flow c. c.	Number trees	Average unit flow c. c.
1. Union healed .....	53	8.79	41	3.94
2. Scion lip not healed .....	9	4.49	60	3.78
3. Stock lip not healed .....	3	3.65	11	2.55

Taking up first the data for the healthy trees, it is interesting to note the large number of trees in class one as compared with those in classes two and three. Out of a total of 65 healthy trees, 53, or approximately 81 percent, showed perfect healing of the union; nine and three trees, or approximately 14 and 5 percent respectively, showed an imperfectly healed union. It is evident from the average unit flow of the trees in classes two and three that the imperfectly healed union caused a serious reduction in water conduction through the healthy tree, amounting to approximately 51 percent.

In galled trees where the union was completely healed the presence of a gall caused a serious reduction in water flow. Comparing the galled trees in class one with the healthy trees of the same class, we find their respective unit flow values were 3.94 c.c. and 8.79 c.c. Thus in these trees the presence of a gall on the otherwise normal union interposed an obstruction which reduced the water flow about 55 percent. In class two, where the scion lip had not entirely healed, and there was a break in the continuity of the vessels, the unit flow was 3.78 c.c. If we look at the unit flow for this class among the healthy trees we note that it was 4.49 c.c., a little higher than for the galled trees. We find also that the incomplete healing of the scion lip in the healthy trees caused a reduction of approximately 33 percent in the water flow through the union, indicating clearly that it was not necessary to have a gall in order to reduce the flow through the union.

This leads us to a consideration of the effect of the gall on a tree where the union is imperfect due to poor grafting. If we take the group in which the scion lip had failed to unite with the stock, we note that the difference in rate of flow between galled and healthy trees was only nominal, less than 16 percent. Then the question at once arises as to the position of the gall on trees of this class. Should they occur on the tip of the scion lip, the effect of flow would probably be negligible. On the other hand, where the gall occurred at some other point on the union the rate of flow would obviously be reduced as shown in the trees where the union healed normally with a gall at some point on the union. In compiling the group in which the scion lip was not healed, there is no way of ascertaining exactly the position of the galls. Allowing that approximately 75 percent of the galls occurred on the scion lip, the difference in reduction in rate of flow between the galled and healthy trees in this class would be caused by the remaining 25 percent at some other point on the union.

It appears from the above evidence that the practice of grafting in many cases results in as serious a curtailment of the water conducting capacity of the young tree as the presence of a gall. The retardation in water flow due to an imperfect union must certainly be reflected in the subsequent growth of the tree for a period of years.

Another point of interest is the number of galled trees falling into the two classes of trees with imperfect unions. Out of a total of 112 galled trees 71 showed an imperfect union.

Hedgcock (1910) makes the following statement in this connection: "A poor fitting of root grafts also causes an increase of abnormal callus, permitting a greater communicability of crown gall during the first year's growth." In his experiments, of a total of 357 trees grown from smoothly fitted root grafts, 7.8 percent were galled, while from 217 trees grown from poorly fitted grafts 17.5 percent were galled. Melhus and Maney (1921) in

their experiments planted perfect and poorly fitting piece-root apple grafts and conclude from an examination of the three-year-old trees that the method of grafting is not so important a factor in crown gall infection as previously considered. They maintain that in those grafts in which the stock lip projected over the base of the scion, the stand at the end of three years was reduced 50 percent in comparison with the stand of trees from perfectly fitting grafts. At the end of three years, only half of the trees from this type of graft were living. These results are readily explained when we consider the reduction of 51 percent in rate of flow through the imperfect union of healthy trees, as shown in table XII. It is suggested from the foregoing field observations and laboratory trials that trees from such grafts cannot obtain sufficient food material due to the lack of continuity of the vessels through the imperfect union; dwarfing or death results.

It seems evident from this study on the extent of healing of the union in relation to water flow that an imperfectly healed union offers, for a time at least, as much interference to conduction as the presence of a gall. The fact that a greater number of trees with imperfect unions show galls is also suggestive. Histological studies on such trees reveal the fact that many of these galls are caused by the formation of excessive callus tissue from the tip of the scion and stock lips. Failure to obtain *Ps. tumefaciens* from such galls, while not proving the absence of the pathogen, is strongly indicative of their origin from the callus tissue at the union.

The results of this study also indicate that the loss in stand of grafts during the first season might be materially reduced by more careful attention to the making of grafts with perfectly fitting unions. This would eliminate excessive callus and discontinuity of the water conducting vessels.

At this point one naturally wonders what may be the condition when trees with such abnormalities are set in an orchard. The trees used constituted a part of those dug by Greene and Melhus (1919) in their study on the effects of crown gall upon young apple trees after two to eight years in the orchard. Although only a few trees were available, the overgrowth was located at the tip of the scion lip, meaning that it had failed to unite with the stock, inducing, therefore, a lack of continuity through the union. This is expressed by Greene and Melhus (1919) as a reduction in twig length, diameter and weight. This correlation of lack of continuity with actual growth on young trees from two to eight years in the orchard makes it worth while to see the actual condition in older trees. Such an opportunity was afforded in an orchard which had been set for 13 seasons\* and dug in the fall of 1924.

The trees were all dug with spades to prevent any breakage of roots or trunks incident to pulling with a tractor or blasting. Records were made of the occurrence of overgrowths on the trees, their location and the amount of heart rot in trunk and roots. The top was removed, leaving a portion of the trunk about 3 feet long and a spread of roots about 2 feet in diameter. These specimens after storage at the nursery over winter were shipped to the laboratory for further study. At the laboratory each specimen was sawed longitudinally, splitting the union into two equal portions, as nearly

\* This orchard was planted in the spring of 1912 by the Iowa Experiment Station and consisted of two-year-old cutback piece-root grafted trees as follows: Wealthy, 126 normal and 200 galled; Jonathan, 120 normal and 210 galled. At the time of setting, no attempt was made to isolate *Ps. tumefaciens* from the galled trees, but selections for planting were made from those showing the largest overgrowths at the union,

as possible through the scion lip. The split specimens were examined critically with a view to determining the relation of the overgrowth at the union to the degree of continuity. Here lack of healing was evidenced by a definite break between the stock and scion. This line of demarcation in some cases was quite pronounced, while in others it was less so. Those specimens were discarded which were improperly split or in which there was doubt as to union or lack of union. For the laboratory studies of the split trees, two varieties were represented; 51 Wealthy, 19 normal and 32 galled; and 44 Jonathan, 18 normal and 26 galled. The number of trees, normal or galled when set in 1912, which showed healing or lack of healing of the union and the presence or absence of gall when dug in 1924, are presented in tables XIII and XIV.

As shown previously (table XII), apparently normal two-year-old trees of mixed varieties may show lack of healing of the union in as much as 17 percent of the cases. Such trees must be regarded as abnormal and it seems entirely possible that an overgrowth at the point of discontinuity at the union might later develop. Should the break between stock and scion be only a slight one, for example at the extreme tip of the scion lip, no overgrowth would probably be produced. That this condition actually develops in the orchard is shown by the data in table XIII. In the 19 normal Wealthy trees there are 10 in which the scion lip had not entirely healed and 6 showing an overgrowth at this point. Likewise, of the 18 Jonathan trees, 8 showed discontinuity at the union and overgrowths on 4 of them.

TABLE XIII. HEALING OF UNION AND PRESENCE OF GALL IN 13-YEAR-OLD TREES NORMAL WHEN PLANTED.

Variety	Number trees examined	Gall		Union healed	Scion lip not healed	Stock lip not healed	Neither lip healed
		Present	Absent				
Wealthy ....	19	6	13	8	10	1	0
Jonathan ...	18	4	14	10	8	0	0

In only one case, in the Wealthy trees, was there lack of healing of the stock lip with the scion, thus causing an overgrowth at this point. It was shown in table XII that 63 percent of the galled trees also showed lack of continuity through the union. Evidence was obtained showing that such overgrowths persisted in old orchard trees.

TABLE XIV. HEALING OF UNION AND PRESENCE OF GALL IN 13-YEAR-OLD TREES GALLED WHEN PLANTED.

Variety	Number trees examined	Gall		Union healed	Scion lip not healed	Stock lip not healed	Neither lip healed
		Present	Absent				
Wealthy ....	32	20	12	8	22	1	1
Jonathan ...	26	22	4	6	17	0	3

In the trees which were galled when planted (table XIV) approximately 60 percent of the Wealthy and 84 percent of the Jonathan showed overgrowths at the union after 13 years in the orchard. Of the Wealthy trees, 22 showed lack of healing of the scion lip, and 20 an overgrowth at this point. Overgrowths were present at the union on 22 of the Jonathan trees, 17 showed lack of continuity between the scion lip and the stock and in 3 the stock lip did not heal. Of the eight Wealthy trees in which the union had healed, six showed no overgrowths, while in the Jonathan five of



the six trees with the union healed showed an overgrowth at the union. In such cases where overgrowths occurred on the perfectly healed union, no attempt was made to determine their cause, but either wound callus or infection from *Ps. tumefaciens* could have been responsible for them.

If the presence of an overgrowth at the union offered an obstruction to the upward passage of food materials, then this effect should be evidenced by some abnormal development in trunk or top growth. Such evidence was found in 36 percent of the Wealthy and 20 percent of the Jonathan trees showing overgrowths at the union. In these trees there was a very evident concavity in the trunk immediately above the gall, which extended upward 10 to 18 inches. This depression was similar to that found immediately above the junction of two lateral roots with the main root.

The significant facts brought out by these studies are, that such overgrowths at the union of young piece-root grafted trees are directly associated with lack of continuity of the scion and the stock; they cause a marked reduction in water conduction through the tree, resulting in a reduction in twig length, diameter and weight; these overgrowths persist and lack of continuity at the union is still evident in 13-year-old orchard trees.

From the above facts it seems obvious that the presence of an overgrowth at the union of orchard trees is indicative of an obstruction to the upward flow of food materials which is reflected in reduction of growth as compared with normal trees.

#### SUMMARY

Cultural studies of 196 piece-root grafted apple trees discarded at the nursery because of overgrowths at the union showed the presence of *Ps. tumefaciens* in relatively few cases. Employing the early method of isolation, the organism was recovered from seven out of 111 trees collected in 1924. Employing Patel's modification of this method on 85 trees, collected in 1925, *Ps. tumefaciens* was recovered in 16 cases.

From 21 trees, grown on an area from which severely galled roses had been dug two years previously, the crown gall organism was isolated in 16 cases. Reisolations from this lot of trees after five months in storage when the galls had begun to decay were negative. However, inoculations from suspensions of the macerated galls yielded typical galls on tomato plants in 15 of the 21 trials.

From many of the overgrowths organisms were found closely resembling *Ps. tumefaciens* in plate culture, but which upon inoculation into tomato failed to produce galls.

The abundant development of fibrous roots from an overgrowth at the union of piece-root grafted trees is not a reliable index of crown gall infection. In the isolations from 196 trees with overgrowths at the union, 155 showed this type of malformation. The crown gall organism was recovered from 16 out of the 155 trees studied.

Isolations from 50 one-year-old apple seedlings showing the fibrous type of hairy root failed to yield the crown gall organism in any case. Inoculations from the suspensions of macerated tissue employed in making these cultures also failed to produce galls upon young tomato plants.

Overgrowths closely resembling those found on discarded nursery trees have been induced upon aseptically made grafts and scion cuttings planted in sterilized soil. Isolations from these malformations failed to yield



*Ps. tumefaciens*. It seems evident that these overgrowths were caused by excess callus formation at the tip of the scion lip of the graft and scion cuttings and it is indicated that a majority of the malformations at the union of piece-root grafted apple trees are due to the same cause.

The fibrous type of hairy root developed on seedlings grown from surface disinfected seed in steamed and unsteamed soil in the field. The fibrous form of hairy root also occurred on seedlings from surface disinfected seed grown in artificially infested steamed soil and under natural field conditions.

The "woolly knot" form of hairy root occurred on seedlings from disinfected seed grown on steamed soil when *Ps. tumefaciens* was artificially inoculated into wounds, or when the seedlings were wounded and the soil was infested with the pathogen. Woolly knot also occurred on wounded and unwounded seedlings in unsteamed soil either artificially infested or under natural field conditions.

The fibrous type of hairy root is probably non-infectious. The "woolly knot" form is a manifestation of crown gall infection.

*Ps. tumefaciens* retains its virulence for considerable periods of time in sterilized and non-sterilized soil. Infection was produced on successive crops of young tomato plants grown in non-sterilized greenhouse soil 102 days after its infestation. In cultures of non-sterilized greenhouse soil 154 days old, the crown gall organism was viable and infectious. Likewise, in 122-day-old cultures of non-sterilized clay soil the organism produced infection on young tomato plants. *Ps. tumefaciens* retained its virulence in twig length, diameter and weight; these overgrowths persist and lack of contamination with a mold and 46 days later was non-infectious on tomatoes.

The average unit water flow through the union pieces of healthy piece-root grafted one and one-half and two-year-old Wealthy, Jonathan and Ben Davis apple trees is 53.4, 20.0 and 42.9 percent less, respectively, than that through the trunk pieces of the same trees. The offset in the trunk due to cutting back the yearling trees, for the above varieties, causes a reduction in water flow of 61.5, 44.6 and 7.9 percent, respectively, as compared with that through the trunk pieces.

The average reduction in water flow through the union pieces of galled one and one-half years old Wealthy, Jonathan and Ben Davis trees is 19.8, 16.7 and 35.5 percent. In later tests after vacuum treatment of the specimens, the reduction in unit flow through similar galled union pieces of two-year cutback Wealthy and Salome trees was 69.7 percent and 21.7 percent, respectively; in two-year-old Jonathan trees the reduction in unit flow was 47.2 percent. Lateral roots arising from above or opposite the gall may counteract the obstruction to water flow caused by the gall. Lateral roots rising from the gall may in some cases balance the reduction in water flow caused by the gall, but such an effect is not constant.

In two-year-old peach trees of the varieties Carman, Salway, Elberta and J. H. Hale, the average reduction in unit water flow caused by the gall is 82.4 percent.

The size of gall and degree of girdling together offer an index of the interference with water conduction through the tree. In approximately 200 galled and healthy two-year cutback apple trees examined, 81 percent of the healthy trees showed a perfect union of scion and stock, and 63 percent of the galled trees showed that the tip of the scion lip had failed to unite

with the stock, causing the development of an overgrowth. The imperfect healing of the union in otherwise normal trees may cause a reduction in water flow equal to that in galled trees.

Lack of continuity between stock and scion was still evident in galled piece-root grafted trees after four, five and eight seasons in the orchard.

On trees showing overgrowths at the union when planted, the malformation persists at least 13 years after setting in the orchard. In a majority of cases such trees show lack of continuity at the union.

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## PLATE I.

(A) Two-year-old piece-root grafted Wealthy apple trees with overgrowths at the union. In the center a normal tree.

These specimens are typical of those employed in the isolation studies and the trials on water conduction.

(B.) Galled and normal two-year-old budded peach trees typical of those employed in the water conduction trials.



## PLATE II.

(A.) Galled apple trees from a lot of 21 grown on infested soil and typical of those from which *Ps. tumefaciens* was recovered.

(B.) Two trees of the same lot as those shown in A. These are typical of those from which the crown gall organism was not recovered.





## PLATE III.

(A.) Wealthy apple scions cut aseptically and stored one month in sterilized sand in the greenhouse. Note the formation of callus tissue at the basal end of the scion.

(B.) Overgrowths at the tip of the scion lip on one-year-old grafts. Grafts made aseptically and grown in steamed soil.



## PLATE IV.

(A.) One-year-old apple seedlings discarded at the nursery because of fibrous hairy root.

(B.) Galls induced on tomato by inoculation with infested soil. From left to right: (1) Inoculated March 26 with sterilized greenhouse soil infested January 12. (2) Inoculated March 18 with unsterilized clay soil infested February 25. (3) Inoculated March 26 with same soil as in (2). (4) Inoculated March 11 with unsterilized greenhouse soil infested January 12.







# THE COLON-TYPHOID GROUP OF BACTERIA AND RELATED FORMS. RELATIONSHIPS AND CLASSIFICATION\*

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THE classification and nomenclature of the organisms of the so-called "intestinal" or "colon-typhoid" group of bacteria are still in an unsatisfactory state. There are included in the group a large number of species of economic importance. This has led to an intensive study of a number of individual organisms, but comparatively few attempts have been made to formulate a complete classification. Among such attempts should be mentioned those of Castellani and Chalmers (1919), who outlined a classification and described a number of species, and of Bergy *et al* (1923, 1925), who have attempted with a measurable degree of success to bring together descriptions of species listed in the literature and to differentiate them. A general review of the group has been given by Winslow, Kligler and Rothberg (1919) and a dichotomous key to most of the species has been prepared by Weldin and Levine (1923).

The present paper embodies a review of the literature on classification of the group, as well as the results of studies of a large number of organisms particularly of the colon subsection. Brief descriptions of some species are given. Keys to their classification and discussion of the validity of the names are particularly stressed. It is expected that this paper will be followed by a more extensive one which will more adequately monograph the group.

## DESIGNATION OF THE GROUP

The first question which arises in a discussion of the generic designation to be used is whether the group should be considered as a single genus (with or without subgenera) or whether sufficient differences exist among the various subgroups to warrant the recognition of a number of genera. There seems to be at the present time considerable diversity of opinion with regard to this question. The committee on classification of the American Society of Bacteriologists (1920) included two genera, *Proteus* and *Bacterium*, in the tribe *Bactereae*. Castellani and Chalmers (1919) distributed the organisms generally recognized as being in the group among 14 genera. Weldin and Levine (1923) included in the tribe *Bacterieae* two genera, *Bacterium* (not known to be plant pathogens) and *Erwinia* (known to be plant pathogens). The former was then subdivided into six subgenera. The classification of Bergy *et al* (1923) is similar to that outlined by Weldin and Levine (1923), except they discarded the generic name *Bacterium* and recognized the six subgenera as of generic rank, adding the genus *Encapsulatus* of Castellani and Chalmers.

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There seems to be little doubt but the colon-typhoid series of organisms constitutes a natural group, and the various species are closely related. In most cases proposed subdivisions of the group have been upon the basis of physiological characters. The propriety of using such characters in the differentiation of genera has been questioned (as by Hall, 1926), though apparently universally accepted for the separation of species. The group, however, contains such a large number of species that some method of subdivision is highly desirable. Such subgroups may be characterized with a considerable degree of satisfaction. The difficulty facing the student is whether to recognize a single genus with subgenera, or to recognize a group of genera.

If the entire group is to be included in a genus, the choice of a generic name is difficult. Strangely enough, apparently no new generic name has ever been proposed for the group as a whole. The only name which has found any degree of general acceptance is *Bacterium*. There is good reason to regard this name as invalid for this group under any strict interpretation of the Code of Nomenclature. It could, of course, be validated by international agreement, if thought desirable. (See discussion of *Bacterium*, p. 128.

There is an increasing tendency, particularly in America (especially since the publication of Bergey's Manual), to recognize a group of genera. This is the procedure here adopted. However, in the discussion of each species, the alternative name, if included in the genus *Bacterium*, is given. It is believed that in course of time the recognition of a number of genera will be common practice.

#### CHARACTERISTICS OF THE GROUP

This group contains a large number of species and varieties, many of which are normally found in the intestinal tract of man and animals; others are found in a variety of habitats. The organisms are all Gram-negative, non-spore-forming short rods. They are rarely found in chains. Involution forms are not uncommon. Some species are motile, the flagella when present being peritrichic. They all grow well upon ordinary laboratory media. The metabolism is complex, amino-acids being utilized and generally carbohydrates. Most of the species produce acid from carbohydrates and some form gas composed mostly of  $\text{CO}_2$  and  $\text{H}_2$ .

It should be noted the line of demarcation between this and some other groups of bacteria is not distinct. Especially is this true with *Pasteurella* forms, whose distinguishing feature is ordinarily given as bipolar staining. Many of the organisms of the colon-typhoid group will, on occasion, exhibit bipolar staining though perhaps not so distinctly as the true *Pasteurella* species. While the members of the intestinal group are not ordinarily considered as pigment formers, some strains, particularly of the colon subsection, exhibit yellow pigmentation even on agar slants.

The following characteristics have been found useful in differentiation of the members of the group: Motility, capsule formation, liquefaction of gelatin, fermentation of carbohydrates, acetyl-methyl-carbinol production, production of indol and hydrogen sulphide, utilization of uric acid as the sole source of nitrogen and citric acid as the sole source of carbon, behavior in litmus milk, agglutination and agglutinin-absorption reactions, and pathogenicity.

## PROPOSED GENERIC AND SUBGENERIC NAMES NOT RECOGNIZED

1. *Actinobacter*. This name was proposed by Duclaux in 1882 for a group of milk bacteria. The first species named was *Actinobacter polymorphum*. The genus was recognized as valid by Maggi (1886). No species have been included in the genus other than the type. From the description it would seem that the organism was probably related to the species usually termed *Aerobacter aerogenes* or *Bacterium aerogenes*, (the *Bacillus lactis aerogenes* of many authors). As Buchanan (1925) observes, it is doubtful if the type species could ever be determined from its descriptions and it would, therefore, be inadvisable to accept as valid a genus with such an uncertain type species. It may be considered as a possible synonym of the genus *Aerobacter*. To accept it as adequately described would cause it to replace *Aerobacter* as generic designation.

2. *Alkaligenes*. A variant spelling of *Alcaligenes* used by Castellani and Chalmers (1919).

3. *Balkanella*. Used by Castellani and Chalmers (1919) for the seventh genus of their tribe *Ebertheae*. Definition: *Ebertheae* which ferment glucose completely with the production of acid and gas; lactose not fermented. Milk clotted. Type species: *Balkanella coagulans* Castellani, 1916.

Only three species were listed. The genus has not been recognized by other authors.

The clotting of milk would hardly seem to be of sufficient importance to warrant separation of this group from the genus *Salmonella*.

4. *Brucella*. Meyer and Shaw (1920) proposed this name for a genus to include the organisms causing Malta fever and infectious abortion of cattle. They did not give a generic diagnosis for the genus and considered only the two organisms mentioned.

Evans (1923) discussed the name in detail. She pointed out that Castellani and Chalmers had previously described a genus which they called *Alcaligenes*, whose definition would include the *melitensis-abortus* group, although they did not discuss these organisms. She was, herself, of the opinion that a generic distinction should be made between *Alcaligenes* and *Brucella* on the ground that the organisms included in the former are characteristically saprophytes, while the *Brucella* types are characteristically tissue invaders. The latter are also slightly different morphologically, being somewhat smaller and producing great numbers of coccoid cells. She gave a general description of *Brucella melitensis*, the type species for the genus.

The morphological dissimilarities between *Brucella* and *Alcaligenes* are hardly definite enough to serve in themselves for differentiation. It is true that as regards habitat and pathogenicity, *Br. abortus* and *Br. melitensis* are distinct from the *Alcaligenes* organisms. Bacteriologists have in general avoided these characters for generic differentiation. It should be noted that Evans would include in the genus an organism (*Bacillus abortus* var. *lipolyticus* Evans 1916), which she isolated from freshly drawn cow's milk and for which no pathogenicity is recorded. On the whole, it is felt that no sufficiently conclusive reasons for the creation of a genus *Brucella* have yet been advanced. The organisms suggested as belonging to it will be considered as members of the genus *Alcaligenes*.

5. *Cloaca*. Castellani and Chalmers (1919) used the name *Cloaca* for the second genus of their tribe *Proteae*, a tribe distinguished from the rest of the non-spore-forming, Gram-negative rods by not producing pigment and by liquefying gelatin. The genus was defined as: "Slow gelatin liquefiers; ferment lactose; Gram-negative." Type species was *Cloaca cloacae* (Jordan, 1890). Only one other species (*Cloaca levans*) was listed.

Weldin and Levine (1923) included these species in their subgenus *Aerobacter* and Bergey *et al* (1923) in the genus *Aerobacter*.

Since liquefaction of gelatin by these organisms is frequently slow and relatively difficult to recognize, and since their characters conform to the definition of the genus *Aerobacter*, it seems best to include them with the latter.

6. *Dysenteroides*. Used by Castellani and Chalmers (1919) for the fourth genus of their tribe *Ebertheae*. Definition: *Ebertheae* partially fermenting glucose and lactose, with the production of acid, but no gas. Milk not clotted. Type species: *Dysenteroides metadysentericus*.

The genus has not been recognized by other authors.

All motile forms which ferment glucose with acid but not gas are sufficiently related to be in a single genus. To break up this group into three genera on the basis of acid production from lactose and clotting of milk is considered unwarranted. Castellani and Chalmers differentiated the genus *Dysenteroides* from their genus *Eberthus* on its ability to produce acid from lactose and further, from their genus *Lankoides* by its inability to coagulate milk. These characters are considered inadequate for generic differentiation. The members of this genus will be included here among those of the genus *Eberthella*.

7. *Eberthus*. The name given by Castellani and Chalmers (1919) to the second genus of their tribe *Ebertheae*. It was described as follows: *Bacillaceae* motile, partially fermenting glucose with the production of acid but no gas. Lactose not fermented. Milk not clotted. Type species: *Eberthus typhosus* (Zopf, 1885).

This definition conforms in large part to that given for the *Eberthella* group; the type species used is the same. What has been said with regard to *Dysenteroides* applies equally well here. The genus *Eberthus* is included, therefore, in the genus *Eberthella*, which has priority.

8. *Encapsulatus*. This term was first used by Castellani and Chalmers (1919) for the single genus of their tribe *Encapsulateae*. Its characters were those of the tribe, which were given as follows:

*Bacillaceae* growing well on ordinary laboratory media without endospores, neither fluorescent nor chromogenic aerobes, not liquefying gelatin, possessing capsules in animal tissues.

The type species of their genus was *Encapsulatus pneumoniae* (Friedlaender, 1883). Besides their type species they included two other organisms, *Encap. acidi lactici* and *Encap. lactis aerogenes*.

Bergey *et al* (1923) recognized both the tribe *Encapsulateae* and the genus *Encapsulatus*, with a somewhat modified characterization, but with capsule formation as the significant character for differentiation from other tribes and genera. Their type species was *Encapsulatus pneumoniae*. They did not, however, include either *Encap. acidi lactici* or *Encap. lactis aerogenes* in the genus.



Perkins (1925) is strongly in favor of a genus *Encapsulata* to include *Bacillus aerogenes*, *B. duodenale* (*B. acidilactici*), *B. pneumonicum* (*B. pneumoniae*), and *B. rhinoscleromae*, all of which he says are constant capsule formers.

Considerable difficulty is foreseen in the use of capsules as a character for generic differentiation. Capsule formation is often demonstrated only with difficulty. The writer knows of no organism which constantly produces evident capsules on all media on which the organism grows. Many of the so-called capsule-formers produce their capsules only when grown in the animal body or upon some special medium such as milk or serum media. *Escherichia acidilactici*, for example, is listed by some authors—notably by Perkins and by Castellani—as producing easily demonstrated capsules. The original descriptions by Hueppe and by Grotenfelt make no mention of capsules, nor do any of the earlier descriptions. MacConkey (1905), a recognizedly careful worker, who considered capsule formation an important character, studied a Kral transfer of the organism and did not anywhere indicate its ability to produce capsules. Perkins (1904) studying a Kral transfer emphasized the presence of capsules. As both of these investigators worked at about the same time, and with presumably the same culture from the Kral laboratory, the natural conclusion is that capsule formation for this organism is at best an indistinct and unreliable characteristic.

By some authors, capsules are considered a purely morphological characteristic of certain organisms, the capsule being considered a thickened cell wall. Buchanan (1924) says, "a thin capsule or layer of capsular material is present in most bacteria, but in only a few does it become very thick and conspicuous." There is a possibility that almost any organism may be made to develop thick capsules if grown under proper conditions. The argument for capsule formation as a basis of differentiation on the ground that capsules are a morphological character thus becomes a contention for the employment of the relative thickness of the cell wall as a fundamental criterion for generic differentiation. If, on the other hand, we take the viewpoint that capsules are a physiological product of certain organisms, a response to certain chemical stimuli, the character possesses no advantage over other more constant and more easily determined physiological characters. In any case, in order to make the character of value, a certain set of conditions should be defined under which all the so-called capsule formers regularly produce capsules and all others of the intestinal group not included in the genus, do not produce them. Such a set of conditions has not been defined to date.

Further, there is considerable evidence that with proper technique all or nearly all the organisms of the colon-typhoid series may be shown to be capsule formers. Marrasini (1913) described a capsulated typhoid bacillus. Fletcher (1918) found mucoid types of *Bact. aertrycke* and of the paratyphoid B organism. Recently Cooper (1925) has demonstrated capsules on cells of "*Bacillus typhosis*, *B. coli*, *B. enteritidis*, *B. dysenteriae-Shiga*, *B. paratyphosus A*, *B. paratyphosus B*, *B. fecalis-alkaligenes*, *B. proteus-vulgaris* and *B. cloacae*."

Indeed, there seem to be no other reasons for grouping the capsulated forms together other than that they have capsules; they do not have a common source, do not agree as to pathogenicity, nor as to their ability to attack certain carbohydrates. It would seem to be fully as logical and much

safer to include them with the subgroups to which they are most clearly related on the basis of other characters.

If the genus were retained its name should be changed to *Klebsiella*. This name was used by Trevisan in 1885 for a genus based upon the same type species as *Encapsulatus*.

9. *Enteroides*. Castellani and Chalmers (1919) use the term *Enteroides* for the ninth genus of their tribe *Ebertheae*. They describe it as follows:

*Ebertheae* which ferment glucose and lactose completely with the production of acid and gas. Milk not clotted. Type species: *Enteroides entericus* (Castellani 1907).

The only character which differentiates this genus from their genus *Escherichia* is the inability to clot milk, a character which perhaps needs some explanation in view of the statement that lactose is completely fermented with production of acid and gas. Clotting of milk is often dependent to considerable extent upon the treatment which the milk has undergone before inoculation and sometimes occurs only when the cultures are heated. It is felt that this character is unreliable, at least without qualifications, for a differential character for genera or subgenera.

The members of the genus *Enteroides* of Castellani and Chalmers will be included here in the genus *Escherichia*.

10. *Erwinia*. Winslow *et al* (Committee S. A. B., 1917) used this term for a generic name with the following description:

Plant pathogens. Growth usually whitish, often slimy. Indol generally not produced. Acid usually formed in certain carbohydrate media, but as a rule no gas.

The type species was later (1920) given as *Erwinia amylovora* (Burrill) Committee.

Weldin and Levine (1923) recognized the genus, separating its members from those of the genus *Bacterium* solely on the basis of pathogenicity for plants, *Erwinia* species being designated as known plant pathogens. Both genera were included in one tribe, *Bactereae*.

Bergey *et al* (1923, 1925) retained the genus, but placed it in the tribe *Erwinieae* with the genus *Phytomonas*, the latter being non-motile or motile with polar flagella, while *Erwinia* species are motile with peritrichous flagella. The only characters given by Bergey *et al* which serve to differentiate *Erwinia* from their tribe *Bacterieae* are those of habitat and pathogenicity.

Pathogenicity is an unreliable and difficult character to use for differentiation. The information at hand indicates that the members of this genus are very closely related to the colon-typhoid group; some of them, perhaps, should be included in that group. Unfortunately, however, the emphasis has been placed by students of the plant pathogens on a different set of characters for these organisms than those used for the intestinal bacteria and it is practically impossible at present to definitely locate them with the latter. Undesirable as pathogenicity may be considered for a differential character, its use is practically unavoidable until further work has been done on the plant pathogens.

Since pathogenicity, then, is to be considered a primary character for subdivision, it appears the logical procedure should be to group these organisms with the other plant pathogens, as Bergey *et al* (1923) have done.

Many of these latter—polar flagellates, pigment producers, etc.—are evidently more closely related to other tribes than to the *Bactereae*. The most expedient plan, then, is to keep them, temporarily at least, in a separate tribe (*Erwinieae*), with the idea that they may be later redistributed to the tribes and genera to which their morphological, cultural and physiological characters more properly allocate them.

11. *Flexnerella*. Castellani and Chalmers (1919) divided their genus *Shigella* into two subgenera, *Flexnerella*, fermenting mannitol, and *Shigella*, not fermenting mannitol. The mannitol-fermenting organisms, associated with bacillary dysentery, do form a comparatively distinct group on the basis of their serological and physiological activities and might be considered a subgenus of the genus *Shigella*. It is felt that mannitol fermentation is not adequate to warrant subdivision into genera.

12. *Graciloides*. Castellani and Chalmers (1919) used this name for the single genus of the tribe *Graciloideae*. The characters were those of the tribe, which were as follows:

*Bacillaceae* growing very slowly and scantily on ordinary and blood media, without endospores or capsules; neither fluorescent nor chromogenic. Type species of the genus: *Graciloides albofaciens* Castellani, 1904. Two species, *albofaciens* and *tardus*, are recognized.

Relative vigor of growth can hardly be considered a reliable character for differentiation, at least unless the composition and character of the medium used is definitely stated. This is, however, the only distinguishing character of the tribe and genus. It is significant to note that Castellani and Chalmers have included the descriptions of the two organisms of this genus in the table entitled "Aerobic asporogenous intestinal bacilli." Evidently they belong with the colon-typhoid series.

The genus is considered insufficiently characterized to warrant its retention. The species listed as belonging to it by Castellani and Chalmers will be included here in the genus *Shigella*.

13. *Klebsiella*. Trevisan (1885) first used this name for a genus with the type species, *Klebsiella crouposa* (*Bacterium pneumoniae-crouposae* Zopf).

Since the pneumobacillus of Friedlander is the type species, this name, as pointed out by Buchanan (1925) has priority over the term *Encapsulatus* used by Castellani and Chalmers (1919) and Bergey *et al* (1923) with the same type species. Bergey *et al* (1925) substituted *Klebsiella* for *Encapsulatus*.

For the reasons discussed under the heading of *Encapsulatus*, this genus is not recognized here.

14. *Lankoides*. Used by Castellani and Chalmers (1919) for the fifth genus of the tribe *Ebertheae*. Their description is as follows:

*Ebertheae* fermenting glucose partially with the production of acid, but no gas; lactose not fermented or only partially, without gas production. Milk clotted. Type species: *Lankoides pyogenes* (Passet, 1902).

What has been said with regard to the genus *Dysenteroides* of Castellani and Chalmers may be said of *Lankoides*. Like the former, it will be

rejected and the organisms listed as belonging to it included among those of the genera *Eberthella* and *Shigella*.

15. *Wesenbergus*. Used by Castellani and Chalmers (1919) for the eighth genus of their tribe *Ebertheae*. It was defined as follows:

*Ebertheae* which ferment glucose completely and lactose partially, producing acid, but no gas. Milk not clotted. Type species: *Wesenbergus wesenbergi* Castellani, 1913.

Three species were included.

It has been the experience of most bacteriologists that organisms of the colon-typhoid group which attack glucose with production of gas produce gas from all other carbohydrates attacked. Sometimes the amount of gas produced is very small and may even be absorbed by the liquid. Nevertheless, if the proper medium be used, the gas production can be demonstrated. It seems best, therefore, until further work is reported on these organisms, to include them with members of the genera *Salmonella*, *Proteus*, etc.

#### KEY TO GENERA

- a. Fermenting glucose with the production of acid or acid and gas.
  - b. Acid and gas formed from glucose.
    - c. Gas formed from lactose.
      - d. Acetyl-methyl-carbinol not produced from glucose; reversion of reaction in 0.5 percent glucose-phosphate, peptone solution negative or very slow; generally not able to utilize uric acid as the sole source of nitrogen nor citric acid as the sole source of carbon.....Genus 1. *Escherichia*.
      - dd. Acetyl-methyl-carbinol produced from glucose; reversion of reaction in 0.5 percent glucose-phosphate-peptone relatively rapid; generally able to use uric acid as the sole source of nitrogen or citric acid as the sole source of carbon .....Genus 2. *Aerobacter*.
    - cc. Gas not formed from lactose.
      - d. Gas formed from sucrose.....Genus 3. *Proteus*.
      - dd. Gas not formed from sucrose.....Genus 4. *Salmonella*.
  - bb. Acid but not gas formed from glucose.
    - c. Motile .....Genus 5. *Eberthella*.
    - cc. Non-motile .....Genus 6. *Shigella*.
- aa. Neither acid nor gas produced from glucose....Genus 7. *Alcaligenes*.

Genus *BACTERIUM* Ehrenberg em. Orla-Jensen.

SYNONYMY. In case it should be decided to include the "colon-typhoid" group in a single genus with the name *Bacterium*, all the generic names discussed in this paper would be considered synonyms in part.

Buchanan (1925) has thoroughly and adequately discussed the validity of the use of the name *Bacterium*. His conclusion is that there is, at present, no strictly valid definition of a genus *Bacterium* with a recognizable type species. Ehrenberg (1828) first used this generic name, naming the single species *Bacterium triloculare*. His description of the organism, how-



ever, was inadequate for its later recognition. Breed, Conn and Baker (1918) suggested that the name be dropped. Buchanan (1925) seemed inclined to agree. Castellani and Chalmers (1919) and Bergey *et al* (1923) have followed the suggestion and split the colon-typhoid group into a number of genera, for none of which have they used the name *Bacterium*.

The Committee, S. A. B. (1920), however, retained the name including it in their list of names recommended for adoption as approved genera. Their description of the genus was as follows:

*Bacterium* Ehrenberg 1828, emended Orla-Jensen (1909), p. 315. Gram-negative, evenly staining rods. Often motile, with peritrichic flagella. Easily cultivable, forming grape-vine leaf or convex whitish surface colonies. Liquefy gelatin rarely. All forms except *Bact. alcaligenes* and the *Bact. abortus* group attack the hexoses and most species ferment a large series of carbohydrates. Acid formed by all, gas ( $\text{CO}_2$  and  $\text{H}_2$ ) only by one series. Typically intestinal parasites of man and the higher animals although several species may occur on plants and one (*Bact. aerogenes*) is widely distributed in nature. Many species pathogenic.

Type species, *Bact. coli* Escherich 1885, p. 518.

The reason advanced by the committee for recommending the adoption of this generic name as well as a number of others, was that, "it seems desirable to preserve in this way a number of generic names which have come into such general use that their abandonment would cause confusion, particularly in dealing with the large number of medical bacteriologists who are not familiar with the principles of botanical taxonomy." An opinion has already been expressed with regard to the advisability of including the whole colon-typhoid group in a single genus. The term *Bacterium* apparently is the only name which has been suggested for such a genus. The name is familiar, and in the minds of a great many bacteriologists, especially since the Committee Report (1920) has, along with the genus *Proteus*, stood for the group of bacteria under discussion. General use has tended more than ever to establish the name and it is possible that its abandonment may result in more bacteriologists using the term *Bacillus* for all rod-shaped organisms regardless of their other characters, morphological or physiological.

Strict adherence to the rules of botanical nomenclature, however, apparently would make it incorrect to use the term without formal adoption by some authoritative body such as an international Botanical Congress. If such were done, and *Bacterium* approved, the diagnosis for the amended genus *Bacterium* to include the whole of the "colon-typhoid" group might well be as follows:

*Bacterium* Ehrenberg, 1828, emended Orla-Jensen, 1909.

Gram-negative, non-spore-forming rods growing well on artificial media. Generally forming acids from carbohydrates and often gas. Non-motile or motile by means of peritrichous flagella. Generally found in the intestinal tract of man and the higher animals; some species widely distributed in nature.

Type species: *Bacterium coli* Escherich, 1885.

Genus 1. *ESCHERICHIA* Castellani and Chalmers, 1919.

SYNONYMY: *Aerobacter* (in part) Beijerinck, 1900; *Enteroides* Castellani and Chalmers, 1919.

Castellani and Chalmers (1919) used this for the tenth genus of their tribe *Ebertheae*, characterizing it as follows:



*Ebertheae* which ferment glucose and lactose completely; milk clotted. Type species: *Escherichia coli* (Escherich, 1886).

This definition would include all of the colon subgroup of the colon-typhoid series with the exception of those organisms which do not coagulate milk. These they placed in the genus *Enteroides*. (See discussion of *Enteroides*, p. 120).

Weldin and Levine (1923) split the colon subgroup or lactose-fermenters into two subgenera, *Escherichia* and *Aerobacter*. Their description of the former was essentially as follows:

Members of the genus *Bacterium* fermenting glucose and lactose with acid and gas. Acetyl-methyl-carbinol not produced from glucose (Voges-Proskauer negative); reversion of reaction in 0.5 percent glucose-phosphate-peptone solution negative or very slow (methyl red reaction positive); not able to utilize uric acid as a source of nitrogen.

Bergey *et al* (1923) used the term for a genus with the following characterization:

Motile or non-motile rods, commonly occurring in the intestinal canal of normal animals. Attack carbohydrates forming acid and frequently acid and gas. Do not produce acetyl-methyl-carbinol. Type species: *Escherichia coli* (Escherich) Castellani and Chalmers.

It is to be noted that in their key to the genera of the tribe *Bactereae*, *Escherichia* is distinguished by gas production in dextrose and lactose, as well as lack of ability to produce acetyl-methyl-carbinol from dextrose. Their complete description, therefore, agrees essentially with that of Weldin and Levine.

We have here a distinct section of the intestinal group of organisms with correlated characters clearly differentiating it from the rest of the colon-typhoid series and making it worthy of generic recognition. The name *Escherichia* is valid and may properly be used for this section.

**Generic Diagnosis:** *Motile or non-motile, Gram-negative, non-spore-forming rods fermenting glucose and lactose with both acid and gas. Do not produce acetyl-methyl-carbinol from glucose (Voges-Proskauer negative); reverse the reaction in 0.5 percent glucose-phosphate-peptone solution very slowly or not at all (methyl red reaction positive); generally not able to utilize uric acid as the sole source of nitrogen. Gelatin not liquefied. Pathogenicity usually slight.*

Type species: *Escherichia coli* (Escherich) Castellani and Chalmers, 1919.

#### KEY TO SPECIES OF THE GENUS *ESCHERICHIA*

- a. Neither acid nor gas formed from sucrose.
- b. Motile.
  - c. Acid and gas formed from dulcitol.
  - d. Acid and gas formed from salicin; indol produced.
    1. *Escherichia coli*.
  - dd. Neither acid nor gas formed from salicin; indol not produced.
    2. *Escherichia vekanda*.
- cc. Neither acid nor gas formed from dulcitol.
  3. *Escherichia grūnthali*.

- bb. Non-motile.
  - c. Acid and gas formed from dulcitol and salicin.
    - 4. *Escherichia enterica*.
  - cc. Neither acid nor gas formed from dulcitol nor salicin.
    - d. Acid and gas formed from adonitol.
      - 5. *Escherichia acidilactici*.
    - dd. Neither acid nor gas formed from adonitol.
      - 6. *Escherichia vesiculosa*.
- aa. Acid and gas formed from sucrose.
  - b. Motile.
    - c. Acid and gas formed from dulcitol.
      - 7. *Escherichia communior*.
    - cc. Neither acid nor gas formed from dulcitol.
      - 8. *Escherichia pseudo-coloides*.
  - bb. Non-motile.
    - c. Acid and gas formed from dulcitol and salicin.
      - 9. *Escherichia neapolitana*.
    - cc. Neither acid nor gas formed from dulcitol nor salicin.
      - d. Indol produced.
        - 10. *Escherichia coscoroba*.
      - dd. Indol not produced.
        - 11. *Escherichia astheniae*.

SPECIES OF *ESCHERICHIA*

1. *Escherichia coli* (Escherich) Castellani and Chalmers, 1919.

Alternative: 1. *Bacterium coli* (Escherich) Lehmann and Neumann, 1896.

SYNONYMY: *Bakterium coli commune* Escherich, 1885; *Bacillus cavicida* Flügge, 1886; *Bacillus C* Booker, 1887; *Bacillus Escherichii* Trevisan, 1889; *Bacillus Schaefferi* von Freudenrich, 1890; *Bacillus coli communis* Sternberg, 1892; *Bacillus coli* (Escherich) Migula, 1895; *Bacterium cavicida* (Brieger) Migula, 1900; *Aerobacter coli* Beijerinck, 1900; *Bacillus coli verus* Ford, 1901; *Bacillus coli communis verus* Durham, 1901; *Bacillus mustelae septicus* Matzschita, 1902; *Bacillus communis* (Escherich) Jackson, 1911; *Escherichia shaefferi* (von Freudenrich) Bergey et al, 1923; *Escherichia cavicida* (Brieger) Castellani and Chalmers, 1919.

This organism was first described by Escherich (1885) under the name *Bakterium coli commune*. He isolated it from the stools of children. He described it as a short rod 1-5 $\mu$  long by 0.3-0.4 $\mu$  wide, with rounded ends, single or in pairs, motile and without spores. It stained well with anilin dyes and was Gram-negative. On gelatin it showed involution forms. It formed white, spreading surface colonies on gelatin and yellowish sub-

surface colonies. On agar and serum the growth was white and on potato yellowish and spreading. Gelatin, agar and serum were not liquefied. It produced sufficient acid in milk to produce coagulation and it fermented dextrose. It was pathogenic for guinea pigs and rabbits when injected intravenously and for guinea pigs when injected subcutaneously.

In a later and more detailed description (1886) he stated that it would grow anaerobically on glucose, but not in milk nor in lactose solution. Its motility was here described as slow and pendulum-like, "langsam pendelnde Bewegungen." Migula (1895) described it as having peritrichous flagella though not as many as the typhoid bacillus and in his *System der Bakterien* (1900) stated that organisms having polar flagella cannot be included in the species.

Migula (1895) noted that "*B. coli* (Escherich)" is undoubtedly a collective species—"Sammelspecies"—which will be separated into its component species as their individual characters are more fully determined. This has been the case; as the use of physiological characters for differentiation have come into use, the species has become more restricted. However, the essential characters used by Escherich have not been changed and the organism may be properly considered as the type species for the group.

*Bacillus schafferi* von Freudenreich. This organism was first described and named by von Freudenreich (1890). He isolated it from spoiled cheese and from potatoes. It corresponded culturally and morphologically with Escherich's *Bact. coli commune* except that it was a little smaller. It was definitely motile; did not show spores and had little resistance to desiccation, heating or the action of antiseptics. It produced acid and gas from "sugars," and grew anaerobically. Milk was not coagulated, though some acid was found in it. It was not pathogenic for guinea pigs. Von Freudenreich distinguished it from Escherich's organism on the basis of its smaller size, greater motility, ability to grow anaerobically in sugar solutions and its lack of pathogenicity.

McConkey (1909) listed *Bacillus schafferi* as being non-motile and many authors have followed his lead in this respect. Bergey *et al* (1923) listed it as motile, differentiated from *Esch. coli* by its failure to coagulate milk, a questionable characteristic for specific differentiation.

The original description was not very complete and did not sufficiently differentiate the organism from *Esch. coli*. Later descriptions have added little of value. The name is considered a synonym of *Esch. coli* (Escherich) Castellani and Chalmers.

*Bacillus cavicida* Flügge. This organism as originally described by Flügge (1886) is practically identical with *Esch. coli* (Escherich) both morphologically and culturally. It was isolated by Brieger (1884) from human faeces. Castellani and Chalmers (1919) described it as not fermenting maltose; there may be some question as to this character since they list it as producing acid and gas from dextrin. There does not seem to be sufficient reason for identifying it as a definite species.

**Specific Diagnosis:** A motile rod 0.4 to 0.6 $\mu$  broad by 1 to 2 $\mu$  long, conforming to the generic diagnosis. Dulcitol and salicin are fermented with acid and gas production; sucrose and adonitol are not attacked. Indol is produced. Litmus milk becomes acid and coagulated, but is not peptonized. Commonly found in the intestinal tract of man and vertebrates generally. Intestinal pathogenic varieties rare. Sometimes pathogenic when associated with other organisms.

2. *Escherichia vekanda* (Castellani) Bergey et al, 1923.

Alternative: 2. *Bacterium vekanda* (Castellani) Weldin and Levine, 1923.

SYNONYMY: *Bacillus vekanda* Castellani, 1917; *Enteroides vekanda* (Cast.) Castellani and Chalmers, 1919.

This organism was isolated by Castellani from cases of enteroides and appendicitis encountered in the Balkans. It was described as a member of the colon-typhoid series; its physiological characters were given in comparative detail. Castellani and Chalmers placed it in their genus *Enteroides*, which was separated from *Escherichia* by inability to clot milk. There may be some skepticism with regard to this character (see discussion of *Enteroides*); an organism which attacks lactose as well as a number of other carbohydrates with production of acid and gas ordinarily would be expected to produce sufficient acid to coagulate milk, although whether it does or does not often depends upon the treatment of the milk before or after inoculation.

Perkins (1925) feels the species should not be recognized, inasmuch as it has been described only by Castellani. This contention, however, is not valid, for a number of investigators, including Savage (1907), Nicoll (1911), Buchan (1910), Stewart (1917) and Azzi (1917), have described organisms which are apparently identical with Castellani's *vekanda*.

**Specific Diagnosis:** A motile rod, similar to *Escherichia coli* in its morphological and cultural characters; conforming to the generic diagnosis. Dulcitol, salicin and adonitol are fermented with acid and gas; sucrose is not attacked. Indol is not produced. Litmus milk becomes acid. Found in the intestines.

3. *Escherichia grüenthalii* (Morgan) Castellani and Chalmers, 1919.

Alternative: 3. *Bacterium grüenthalii* (Morgan) Weldin and Levine, 1923.

SYNONYMY: Das Grünthaler Bacterium, (?) Fischer, 1902; *Bacillus Grünthal* Morgan, 1905; *Bacillus acidi lactici* var *grüenthalii* Levine, 1918; *Escherichia para-Grünthalii* Castellani and Chalmers, 1919; *Bacterium coli* var. *para-Grünthalii* Weldin and Levine, 1923.

Fischer in 1902 isolated from "liver paste" and "liver wurst" which had caused some cases of food poisoning a bacterium, "welches morphologisch sowie culturell von dem *Bacterium coli commune* nicht zu unterscheiden war, aber bei der Verfütterung an Mäuse den Tod derselben unter den Erscheinungen der hamorrhagischen Enteritis herbeiführte." While Fischer's description is not very detailed as to cultural and physiological characters, his organism would seem to be more closely related to the *enteritidis* type than to the colon organism, both because of its apparent pathogenicity and its reversion of the reaction of milk from a definite acidity to a marked alkalinity. Fischer, however, did not name the organism, simply designating it as "das Grünthaler Bacterium."

Morgan (1905) gave in tabular form the characters, presumably, of the same organism, which he calls "*B. Grünthal*." He described it as a motile bacillus, producing on agar and gelatin a creamy, raised, moist, translucent growth, not liquefying gelatin and producing a general turbidity in broth. Indol was produced, acid and curd in milk and acid and gas in glucose, lactose, mannitol, but not in saccharose nor in dulcitol. Mac-



Conkey (1906) added more carbohydrates to the list attacked and still others have been added by subsequent authors.

The species is adequately characterized and has been recognized by most recent systematists. It may easily be differentiated from *Esch. coli* by its inability to ferment dulcitol.

*Escherichia paragrünthali* Castellani and Chalmers. This organism as described by Castellani and Chalmers is almost identical with *Escherichia grünthali* so far as the characters of the two organisms are known. They make the statement that it differs from the latter in fermenting maltose. However, in their description of the Grunthal organism, and in all available descriptions by other authors, including Castellani (1912), no statement is made as to the behavior of the organism in maltose one way or the other. It would be rather startling if *Esch. grünthali*, which is able to produce acid and gas from dextrin, should fail to attack maltose.

Weldin and Levine (1923) used the term *paragrünthali* for a non-motile variety of *Bacterium coli*; Bergey *et al* (1923) used it for a species of *Escherichia*. While it differs but slightly from *Esch. coli*, it differs still less from *Esch. grünthali*, in neither case being sufficiently distinct to warrant specific rank. It will be considered synonymous with *Escherichia grünthali*.

**Specific Diagnosis:** A motile rod, similar in its morphological and cultural characters to *Escherichia coli*, and conforming to the generic diagnosis. Does not produce acid or gas from sucrose, salacin, dulcitol or adonitol. Indol is produced. Litmus milk is coagulated. Originally isolated from liver paste and liver wurst. Frequently found in the intestines of man and animals.

4. *Escherichia enterica* (Castellani and Chalmers) Comb. nov.

Alternative: 4. *Bacterium entericum* (Castellani and Chalmers). Comb. nov.

**SYNONYMY:** *Bacillus coli immobilis* Kruse, Flügge, 1896; *Bacterium coli immobilis* Chester, 1897; *Bacillus Schaefferi* MacConkey, 1909; *Bacillus entericus* (Castellani) Castellani and Chalmers, 1910; *Enteroides entericus* (Castellani) Castellani and Chalmers, 1919; *Escherichia Schaefferi* (MacConkey) Castellani and Chalmers, 1919; *Bacillus coli* var *immobilis* Winslow, Kligler and Rothberg, 1919; *Bacterium* (*Escherichia*) *schaefferi* Weldin and Levine, 1923; not *Bacillus entericus* Ford, 1903; *Eberthella enterica* (Ford) Bergey *et al*, 1923.

This organism was first described by Kruse (1896) under the name *Bacillus coli immobilis*. The only significant difference listed between it and Escherich's *Bacterium coli commune* was the lack of motility. Kruse's name for the organism was trinomial and should be discarded. *Bacterium immobile* was used by Chester (1901) for *Bacillus fluorescens-immobilis* Kruse (1896).

MacConkey (1909) described an organism practically identical with *Escherichia coli* except for motility, giving it the name *Bacillus schaefferi*. This name is invalid because of the previous use for another organism by Kruse. As has been stated before (See *Escherichia coli*), *Bacillus schaefferi* von Freudenreich was distinctly motile. This name cannot be used for the organism under question.

Castellani and Chalmers (1910) used the term *Bacillus entericus* for a non-motile species, otherwise corresponding to *Escherichia coli*. Later



(1919), the name was changed to *Enteroides entericus* and additional description given. Although Ford in 1903 used the name *Bacillus entericus* for an entirely different species, his name does not invalidate *Escherichia enterica* or *Bacterium entericum*.

**Specific Diagnosis:** A non-motile rod, otherwise similar to *Escherichia coli*. Dulcitol and salicin are fermented with acid and gas production; sucrose and adonitol are not attacked. Indol is produced. Its normal habitat is the intestinal tract of man and animals.

5. *Escherichia acidi lactici* (Grotenfeldt) Bergey *et al*, 1923.

Alternative: 5. *Bacterium grotenfeldtii* Migula, 1900.

**SYNONYMY:** *Bacterium acidi lactici* I, Grotenfeldt, 1889; *Bacillus acidi lactici* (Hueppe) Morgan, 1905; *Encapsulatus acidi lactici* (Hueppe) Castellani and Chalmers, 1919; not "Die Milchsäurebakterien" Hueppe, 1884; *Bacterium acidi lactici* Zopf, 1883, 1884; *Bacillus acidi lactici* Zopf, 1885.

Hueppe (1884) has been cited by many as the authority for the species name *acidi lactici*. While the organism which Hueppe isolated from sour milk and described in exceptional detail for his time conforms in most of its characters to the definition of the genus *Bacterium*, his descriptions showed very definitely that he was working with a spore former. In the cells in unstained mounts Hueppe observed, "stark lichtbrechenden" bodies; these could not be stained with his dyes and, he says, resisted heating. "Dass diese stark lichtbrechenden Körperchen, welche keinen Farbstoff annahmen, wirkliche Sporen waren, konnte dann nicht weiter ermittelt werden dadurch, dass kurzes Aufkochen, welches zum Vernichten der lebensfähigen Bacillen ausreicht, nicht genugte alles Leben in diesen Zuckerlösungen zu vernichten, sondern dass sich aus derselben wieder die unzweifelhaften Milchsäurebacillen entwickelten." Zopf (1885), Schroeter (1889), DeToni and Trevisan (1889), Kramer (1892), Sternberg (1892), Migula (1895, 1900) have all, quoting Hueppe, listed spore formation as a character of this organism. Migula (1895) was the first to describe it under the generic name *Bacterium*. Chester (1901) and Lehmann and Neumann (1896), though giving Hueppe as authority for the name, have omitted any reference to spore production, and practically all authors since 1901 have followed their example. If the term *acidi lactici* is to be applied to the organisms described by Hueppe and we assume that he was working with a pure culture, his *Bact. acidi lactici* must be considered to be a spore former.

Hueppe, however, did not use the term *acidi lactici* at all; he referred to his lactic acid producing organisms only by the term "die Milchsäurebakterien."

Apparently the first use of the species name *acidi lactici* was by Zopf (1883, 1884) for an imperfectly described milk-souring organism which he called *Bacterium acidi lactici*. It was isolated from sour milk, sauerkraut, beer mash, old cheese, sugar solutions, etc. Its description is too brief to permit of its later recognition. The organism showed rods, chains and spherical forms. It was a lactic acid producer, but grew best at 50° C., an optimum growth temperature too high for a member of the colon-typoid group. It is probable that Zopf either had an impure culture or a member of the *Lactobacillus* group. A few later systematists (DeToni and Trevisan, 1889; Chester, 1901; Frost, 1903) gave Zopf credit for the

name *acidi lactici*, but their descriptions followed that of Hueppe. Zopf later (1885) used the name *Bacillus acidi lactici* for Hueppe's "milch-saure-bakterien," which he said was different from his own *Bacterium acidi lactici*.

Grotenfeldt in 1889 described an organism which he isolated from sour milk and which he called *Bacterium acidi lactici* I. The organisms were short rods, 1.0-1.4 $\mu$  long by 0.3-0.4 $\mu$  wide, non-motile and non-spore forming. On gelatin plates they produced porcelain-like, white, glistening, round colonies; on agar, a whitish-yellow, thick, pulp-like layer, with gas bubbles; on potato, a grey or greyish-yellow layer. They were facultative anaerobes. In milk, lactic acid was produced, casein precipitated, gas (CO<sub>2</sub>) and alcohol formed. Gelatin was not liquefied.

This description, as far as it goes, is satisfactory for the organism now commonly known as *Bact. acidi lactici*. Winslow, Kligler and Rothberg (1919) cite Grotenfeldt as the author of the specific name *acidi lactici* for non-spore-forming, short rods. We likewise find a *Bacterium acidi lactici* Grotenfeldt in Eisenberg (1891), Kramer (1892) and Chester (1897, 1901). Migula (1900) changed the name to *Bacterium Grotenfeldtii*.

Zopf's prior use of the combination prohibits *Bacterium acidi lactici* being used for Grotenfeldt's organism. It, however, does not invalidate the use of *Escherichia acidi lactici* (Grotenfeldt) Bergey. This would seem to be the proper designation of the species under discussion, with the alternative of *Bacterium Grotenfeldtii* Migula.

Castellani and Chalmers (1919) list this organism in their genus *Encapsulatus*. Whether Hueppe or Grotenfeldt is considered as having first described the species, neither of the authors listed capsule formation as one of its characters, nor have any subsequent authors with the exception of Perkins (1904) and Castellani and Chalmers (1919). It is very probable that capsules are produced, this characteristic being quite common for many if not all members of the "colon-typhoid" group under suitable conditions, but the fact that very few of the authors who have used the term noted capsule formation would indicate that this characteristic is not evident under ordinary conditions.

**Specific Diagnosis:** A non-motile rod, 0.3 to 0.4 $\mu$  broad and 1.0 to 1.4 $\mu$  long, conforming to the generic description. It produces acid and gas from adonitol but fails to ferment sucrose, salicin or dulcitol. Indol is produced. Litmus milk is acidified and coagulated. Has been isolated from milk, cheese and faeces.

6. *Escherichia vesiculosa* (Henrici) Castellani and Chalmers, 1919.

Alternative: 6. *Bacterium vesiculosum* Henrici, 1894.

SYNONYMY: *Bacillus vesiculosus* MacConkey, 1909; *Escherichia vesiculosa* (Henrici) Bergey *et al.*, 1923.

Henrici (1894) described an organism which he isolated from cheese under the name *Bacterium vesiculosum*. It was a short rod, single or in pairs, sometimes in short chains, non-motile. Growth on gelatin was slimy and dirty-white in color, later becoming brown. On agar the growth was slimy, smooth and glistening. Broth was turbid, with a white sediment and gas production. The organism was aerobic and facultative. There is nothing in the description which might serve to identify the organism on later isolation.

MacConkey (1909) adopted the name for an organism isolated by him from various sources and gave a comparatively complete description of its physiological characters. Perkins (1925) states that in so doing the later author is ascribing characters to an organism which it might or might not have had. That is true enough. However, MacConkey finding his organism identical with that of Henrici so far as the characters are given by the latter, assumed that they were the same and ascribed additional characters to the species. These characters then became amplifications or emendations of the original. This is a logical and recognized procedure and the emended description may be properly accepted as valid for the species in question providing no pure cultures of the original strain have been preserved and proved to have other characters.

**Specific Diagnosis:** A non-motile rod, conforming to the generic description. Sucrose, dulcitol, salicin and adonitol are not attacked. Indol is formed. Acid and curd are formed in milk. The organism was first isolated from cheese, but is common in the intestinal tract.

7. *Escherichia communior* (Durham) Bergey et al, 1923.

Alternative: 7. *Bacterium communior* (Durham) Holland, 1920.

SYNONYMY: *Bacillus coli communior* Durham, 1901; *Bacillus communior* Ford, 1903; *Bacillus paraentericus* (Cast.) Castellani and Chalmers, 1910; *Bacillus pseudo coli* Castellani, 1912; *Escherichia pseudo-coli* Castellani and Chalmers, 1919; *Bacterium coli communior* (Durham) LeBlaye and Guggenheim, 1914; *Escherichia meta-coli* Castellani and Chalmers, 1919; *Escherichia pseudo-coliformis* Castellani and Chalmers, 1919; *Enteroides para-entericus* (Cast.) Castellani and Chalmers, 1919.

Durham (1901) isolated a coli-like organism from animal faeces, which he called *Bacillus coli communior*. He says of it, "Characters and morphology like those of group *Bacillus coli communis verus* except that sucrose is fermented and acid freely formed from it. Mutual serum reactions not frequently met with within the group. I am inclined to think that this is a commoner inhabitant of human faeces than members of the last group, but have not made any direct experiments. Should this prove to be the case, it might be distinguished from the *Escherich* type as *B. coli communior*."

Subsequent investigators have agreed with Durham as to the existence of a large group of organisms in faeces, differing from *Bact. coli* of *Escherich* in ability to attack sucrose and the species has been generally accepted. Durham's name, however, being a trinomial, was not valid. It was shortened to *Bacillus communior* by Ford (1903).

**Specific Diagnosis:** Morphologically and culturally like *Escherichia coli*. Motile. Sucrose and dulcitol fermented with acid and gas production; adonitol not attacked. Indol is formed. Litmus milk is acidified and coagulated. Found in the intestines of man and animals.

8. *Escherichia pseudo-coloides* Castellani and Chalmers, 1919.

Alternative: 8. *Bacterium pseudo-coloides* (Castellani and Chalmers) Weldin and Levine, 1923.

According to Castellani and Chalmers (1919) this organism is identical with their *Escherichia pseudo-coli* (see *Esch. communior*) except that it fails to ferment dulcitol. Weldin and Levine (1923) included the organ-

ism as a species of their genus *Bacterium* and Bergey *et al* (1923) also accepted it as a recognized species.

If Castellani and Chalmers had been the only ones to describe such a species, there would hardly be justification for differentiation on the basis of a single character such as dulcitol fermentation. Quite a long list of unnamed organisms has been found, however, which are identical with *pseudo-coloides* as described by Castellani and Chalmers. Evidently organisms of this type are fairly widespread. It is felt the species should be recognized. A partial list of the unnamed organisms referred to is as follows:

Cathcart (1906) Organism No. 1.

Savage (1907) Organisms Nos. 7, 20, 30, 46 and 60.

Bergey and Deehan (1908) *Bacillus* No. 18, No. 20 and No. 116.

MacConkey (1909) *Bacillus* No. 100, No. 106, No. 109.

Buchan (1910) Organisms Nos. 5, 10, 15, 21, 24, 26, 29, 38, 43, 50, 55, 57 and 58.

Nicoll (1911) *Bacillus* No. 106, No. 109.

Kligler (1914) *Bacillus communior* No. 36.

Rogers, Clark and Davis (1914) Cultures m, bl, bv, bw, cl, cu, ez, dy and aj.

Logan (1914) Organisms Nos. XI 2, X 20, XLI 1, XLV 3, 4, X 18, XLVI 4.

Azzi (1917) Organisms Nos. 16, 2, 9, 43, 28 and 39.

Nankivell and Stanley (1920) Organism No. 10.

Redman (1922) Organisms Nos. 49, 38, 50 and 61.

**Specific Diagnosis:** A motile rod, conforming to the generic diagnosis. Acid and gas are produced from sucrose and generally from salicin and adonitol; dulcitol is not fermented. Indol is usually formed. Acid and curd are formed in litmus milk. Found in the alimentary tract of man.

9. *Escherichia neapolitana* (Flügge) Castellani and Chalmers, 1919.

Alternative: 9. *Bacterium neapolitanum* (Flügge) Lehmann and Neumann, 1896.

**SYNONYMY:** *Bacillus neapolitanus* Flügge, 1886; *Escherichia neapolitana* Bergey *et al*, 1923.

This organism was first described by Emmerich (1884). He isolated it from cholera cadavers in Naples and once from the blood of a patient and thought it to be the cause of cholera in man. Other investigators soon showed that he was mistaken. Emmerich described it as a short rod with rounded ends, single or in pairs, about one and a half times as long as broad, non-motile. It grew on gelatin as an opalescent layer, deep colonies a yellowish brown; gelatin was never liquefied. On agar, a moist white layer; on potato, yellowish brown. It was aerobic and facultative, non-spore-forming and Gram-negative. It was pathogenic for laboratory animals when injected in large amounts. Emmerich called the organism the "Neapel bacillus." Flügge (1886) seems to have been the first to give it a specific name, *Bacillus Neapolitanus*.



There is little in Emmerich's description, or in the descriptions given by any author up to 1905 (Flügge, 1886; Trevisan, 1889; Eisenberg, 1891; Lehmann and Neumann, 1901, etc.), which might serve to identify the organism. MacConkey, however, working with a culture obtained from Kral, studied its action on a number of carbohydrates and added sufficient information in this respect definitely to differentiate it from related species. He separated it from *Bact. coli commune* Escherich on its non-motility and ability to produce acid and gas from cane sugar. Its characters have been studied in still greater detail by subsequent investigators until today it stands as a well-defined and easily recognized species.

**Specific Diagnosis:** A non-motile rod, conforming to the generic diagnosis. Acid and gas are produced from sucrose, dulcitol and salicin, but not from adonitol. Indol is produced. Litmus milk is acidified and coagulated. Found in the intestinal tract of man.

10. *Escherichia coscoroba* (Tretop) Comb. nov.

Alternative: 10. *Bacterium coscoroba* (Tretop) Holland, 1920.

**SYNONYMY:** *Bacillus coscoroba* Tretop, 1900; MacConkey, 1906; *Bacillus communior* var. *coscoroba* Winslow, Kligler, Rothberg, 1919; *Escherichia pseudocoscoroba* Castellani and Chalmers, 1919.

Tretop (1900) has generally been given credit for the term *coscoroba*. He used it for an organism which he had isolated from diseased swans. Castellani and Chalmers (1919) contended that his organism was not a member of the colon-typhoid series, but belonged rather to the *Pasteurella* group. Tretop did, indeed, emphasize polar staining—"les coccobacilles des organes retiennent fortement la matière colorante aux poles, le centre restant clair"—and the pathological symptoms noted in swans dead of the disease correspond closely to those ordinarily given for hemorrhagic septicemias. No mention was made of its ability to attack any carbohydrate. The evidence, so far as Tretop's description goes, perhaps does favor assignment to *Pasteurella*.

However, MacConkey (1906) studied an organism labelled *B. coscoroba* which he had secured from Dr. Binot at the Pasteur Institute and which presumably was a subculture of Tretop's bacillus. He found it to be a non-motile rod, which fermented (with acid and gas production) glucose, lactose, sucrose, galactose, laevulose, mannose, arabinose, raffinose, mannitol, and did not liquefy gelatin. Indol was produced. Whether or not this organism was really a lineal descendant of Tretop's bacillus, of course, cannot be proven. Such evidence as we have does not indicate that it was not. Polar staining has often been described for members of the colon-typhoid series. If it could be shown that MacConkey was mistaken in identifying his organism with that of Tretop's, the name *pseudocoscoroba* proposed by Castellani and Chalmers (1919) would replace *coscoroba*. Until this is shown, however, *Bacillus coscoroba* Tretop emended MacConkey must be considered a valid species, clearly a member of the "colon-typhoid" group.

**Specific Diagnosis:** A non-motile rod, conforming to the generic description. Sucrose is fermented with acid and gas; dulcitol, adonitol and salicin are not attacked. Indol is produced. Litmus milk becomes acid and coagulated. Originally described from an epidemic of swans. Found in the intestinal tract and in sewage.



11. *Escherichia astheniae* (Dawson) Bergey *et al.*, 1923.Alternative: 11. *Bacterium astheniae* Dawson, 1898.

Dawson (1898) described this organism briefly as follows: A rod, 1-1.3 $\mu$  long and 0.5 $\mu$  wide, often in pairs. Does not stain with acid or alkaline methylene blue, carbol fuchsin, or any alcoholic solutions of stains. Stains well with aqueous solutions of fuchsin, methylene blue, Bismarck brown, night blue and Gram's stain. Aerobic and facultative anaerobic. Bouillon, turbid, with pellicle and ring on glass; putrefactive odor and yellowish green color. Produces acid and gas (H<sub>2</sub> and CO<sub>2</sub>) from glucose, lactose and sucrose. Coagulates milk, with acid and whey. Growth on gelatin, spreading, brownish, deeply dentated. Agar growth, luxuriant, white, opaque, with wavy margins. Potato growth, yellowish, creamy, spreading, with gas containing blisters, and pungent, disagreeable odor. Does not produce indol nor phenol. Isolated from duodenal contents of chickens in an asthenic condition. Pathogenic for guinea pigs and rabbits.

The organism was included by Weldin and Levine (1923) in the genus *Bacterium* (subgenus *Escherichia*) and subsequently by Bergey *et al.* in the genus *Escherichia*.

The fact that it is Gram-positive would tend to throw it out of the colon-typhoid series. The production of a yellowish green pigment in acid bouillon might indicate the genus *Pseudomonas* as its proper group; the members of this genus, however, should also be Gram-negative. In fact, there is no described genus to which it might properly belong. Perkins (1925) suggests the organism was really Gram-negative. Its other characters, namely ability to attack carbohydrates, its normal habitat and its cultural and morphological characters, show it to be more closely related to the colon-typhoid organisms than to any other recognized group, and it seems, therefore, that it might be included tentatively with them.

**Specific Diagnosis:** A non-motile rod, reported Gram-positive, but otherwise conforming to the generic diagnosis. Ferments sucrose with acid and gas production; does not attack dulcitol nor salicin. Indol is not produced. Litmus milk is acidified and coagulated. Isolated from the intestines of chickens in "asthenia."

Genus 2. *AEROBACTER* Beijerinck 1900, Emend. Weldin and Levine, 1923.

**SYNONYMY:** *Actinobacter* (?) Duclaux, 1882; *Cloaca* (in part) Castellani and Chalmers, 1919; *Encapsulatus* (in part) Castellani and Chalmers, 1919.

The name *Aerobacter* was used by Beijerinck (1900) to designate a part of the so-called "colon" group. The organisms of his genus were considered to be facultative anaerobes in sugar solutions. They fermented dextrose and levulose with production of acid and usually of gas (CO<sub>2</sub> and H<sub>2</sub>). Sulphates were never reduced. Nitrates were reduced to nitrites, but not to ammonia. No spores were found. Flagella, when present, were either peritrichous or monotrichous. In a list of organisms included in the genus the first was *Aerobacter aerogenes* (*Bacillus lactis aerogenes* Escherich). While Beijerinck's genus would include many more organisms than are included, for instance by Bergey *et al.* (1925) under the same name, his description is not broad enough to take in the whole "colon-typhoid" group.

Buchanan (1918) used the term for a subgenus of the genus *Bacterium*. He included all the organisms of the colon-typhoid group which produce gas from lactose, erroneously giving *Bacterium* (*Aerobacter*) *coli* Escherich

as the type. Enlows (1920) recognized the genus with a definition closely resembling the original definition of Beijerinck. Weldin and Levine (1923), using *Aerobacter* as a subgeneric name, restricted it to the lactose-fermenters which produce acetyl-methyl-carbinol from glucose. While they did not definitely designate a type species, they listed *Bacterium aerogenes* first in their key to species.

Bergey *et al* (1925) used the name for the twelfth genus of the family *Bacteriaceae*. The genus as characterized by Weldin and Levine (in subgeneric sense) is clearly differentiated from other subdivisions of the colon-typhoid group upon correlated characters. *Aerobacter* is apparently the valid name if the group is to be accorded generic recognition.

**Generic Diagnosis:** Motile or non-motile, Gram-negative, non-spore-forming rods, fermenting both glucose and lactose with both acid and gas. Produce acetyl-methyl-carbinol (Voges-Proskauer reaction positive); reverse the reaction of 0.5 percent glucose-phosphate-peptone solution relatively rapidly; generally able to utilize uric acid as an available source of nitrogen. Pathogenicity usually slight or absent.

Type species: *Aerobacter aerogenes* (Escherich, 1885), Beijerinck, 1900.

#### KEY TO SPECIES OF THE GENUS *AEROBACTER*

- a. Non-motile. (Acid and gas formed from glycerol; starch, adonitol and inositol usually fermented with acid and gas; gelatin rarely liquefied.)
  - b. Acid and gas formed from sucrose.
    - c. Neither acid nor gas formed from dulcitol.
      1. *Aerobacter aerogenes*.
    - cc. Acid and gas formed from dulcitol.
      2. *Aerobacter oxytocum*.
  - bb. Neither acid nor gas formed from sucrose.
    3. *Aerobacter chinense*.
- aa. Motile. (Glycerol, starch, adonitol and inositol rarely fermented; gelatin usually liquefied.)
  - b. Acid and gas formed from sucrose.
    4. *Aerobacter cloacae*.
  - bb. Neither acid nor gas formed from sucrose.
    5. *Aerobacter levans*.

#### SPECIES OF *AEROBACTER*

1. *Aerobacter aerogenes* (Escherich) Beijerinck, 1900.

Alternative: 12. *Bacterium aerogenes* (Escherich) Chester, 1897.

SYNONYMY: *Bacterium lactis aerogenes* Escherich, 1885; *Actinobacter polymorphum* (?) Duclaux, 1883; *Bacterium aceticum* Babinsky, 1888; *Bacillus capsulatus* Pfeiffer, 1889; *Bacillus guillebeau* C. Freudenreich, 1890; *Bacillus lactis aerogenes* Sternberg, 1892; *Bacterium capsulatum* Migula, 1895; *Bacillus aerogenes* Kruse, Flügge, 1896; *Bacillus lactantium* Trevisan, 1889; *Encapsulatus lactis aerogenes* (Escherich) Castellani and Chalmers, 1919; *Encapsulatus capsulatus* Castellani and Chalmers, 1919; *Encapsulata aerogenes* Perkins, 1925.

This organism was first described by Escherich in 1885 and again in greater detail in 1886 under the name *Bacterium lactis aerogenes*. Like his *Bakterium coli commune* it was first isolated from the stools of milk-fed children. It was described as a short rod with rounded ends,  $1.4\text{--}2\mu$  long by  $0.5\mu$  broad, usually in groups of two. According to his first description, spores were produced in sugar solution, but in 1886 Escherich says he was evidently mistaken about spore production. It was non-motile, easily stained, but Gram-negative. The colonies on gelatin were round, arched, viscid ("saftig") and glistening; gelatin was not liquefied. On agar it formed a luxuriant white layer; on blood serum a white strip. On potato it formed a yellowish white layer with some gas bubbles. It produced sufficient acid for coagulation in milk, the principal acid being lactic; whey was squeezed from the coagulated casein. Glucose, lactose and sucrose were fermented with acid and gas ( $\text{CO}_2$  and  $\text{H}_2$ ); it grew anaerobically in the presence of sugars. It was considered slightly pathogenic when injected into experimental animals. Escherich found it in intestinal contents of milk-fed animals and children, in the faeces of the same and once in unheated milk.

There seems to be but little question but that the species known today under the specific name "aerogenes" is the same one that Escherich described, though later authors have added much to our knowledge of its physiological characters. Castellani and Chalmers have placed the species in their genus *Encapsulatus*, and Perkins lays great stress on its ability to produce capsules. While Escherich did not definitely give capsule formation as a character of the species, his description of its growth on gelatin suggests it. Many other authors have described capsule formation, especially in milk. As stated elsewhere, however, capsule formation is not considered a desirable character for group differentiation. (See *Encapsulatus*.)

The name was changed by Chester (1897) to *Bacterium aerogenes* to eliminate the trinomial form. Beijerinck (1900) placed it in his newly created genus, *Aerobacter*.

**Specific Diagnosis:** A non-motile rod,  $0.5$  to  $0.8\mu$  broad by  $1.0$  to  $2.0\mu$  long, conforming to the generic diagnosis. Acid and gas are formed from sucrose, glycerol, inositol, adonitol and usually from starch; dulcitol is not attacked. Gelatin is rarely liquefied. Indol is rarely formed. Litmus milk is made acid and coagulated. The organism is found in the alimentary tract of man and animals and widely distributed in nature.

## 2. *Aerobacter oxytocolum* (Flügge) Bergey et al, 1923.

Alternative: 13. *Bacterium oxytocolum* (Flügge) Migula, 1900.

SYNONYMY: *Bacillus oxytocolus perniciosus* (Wyssokowitsch) Flügge, 1886; *Escherichia oxytocolus perniciosus* (Wyssokowitsch) Castellani and Chalmers, 1919.

This organism was described under the name *Bacillus oxytocolus perniciosus* (Wyssokowitsch) in Flügge, Die Mikroorganismen, 1886. Flügge states that it was isolated by Wyssokowitsch, a student in his laboratory. No publication of the name by the latter has been found, and it is presumed that such publication does not exist. Flügge rather than Wyssokowitsch should, therefore, be given credit for the specific name.

The organism was isolated from old milk. It was a short bacillus with rounded ends. On gelatin, deep colonies were small and yellowish; surface

colonies, grayish-white, round and arched. Milk was coagulated with acid reaction in 24 hours. Large doses injected into the veins of rabbits caused severe and fatal diarrhoea.

MacConkey (1906) obtained what was presumably a subculture of the original strain from Kral. He found it able to produce acid and gas from every carbohydrate tested (including glucose, lactose, sucrose and dulcitol) except erythritol. It was Voges-Proskauer positive, did not liquefy gelatin, did not produce indol and was non-motile.

It seems that an organism exhibiting such exceptional fermentative ability should be recognized as a species. Migula (1900) reduced the trinomial to *Bacterium oxytocom*.

**Specific Diagnosis:** Non-motile rods, conforming to the generic diagnosis. Sucrose, dulcitol, glycerol, adonitol and inositol fermented with acid and gas production. Gelatin not liquefied. Indol is usually produced. Litmus milk is acidified and coagulated. Was first isolated from old milk. Found in dairy products, soil and the alimentary tract. Is pathogenic for rabbits on intravenous injections.

### 3. *Aerobacter chinense* (Hamilton) Bergy *et al*, 1923.

Alternative: 14. *Bacterium chinense* (Hamilton) Migula, 1900.

**SYNONYMY:** *Bacillus capsulatus chinensis* Hamilton, 1898; *Bacterium duodenale* (?) Ford, 1903; *Aerobacter chiense* (Hamilton) Bergy *et al*, 1923.

This organism was isolated by Hamilton (1898) from Chinese ink. She described it under the name *Bacillus capsulatus chinensis* as a capsulated rod, 4-6 $\mu$  long by 0.5-0.75 $\mu$  broad, with two or three members in capsule, involution forms common, non-motile, non-spore-forming, Gram-negative. Capsules are more readily formed on nutrient media than in the animal body. On gelatin the colonies were white, glistening, hemispherical with sharp edges; deep colonies a yellowish color. Gelatin was not liquefied. On agar the growth was quicker and more abundant, the colonies appearing as large slimy drops. On glycerin agar and sucrose agar a thick slimy layer covered the whole surface. Blood serum did not support as good a growth as agar; it was not liquefied. On potato the growth was creamy and distinct ammonia odor was produced; at 37° C. gas bubbles were formed and the reaction was distinctly alkaline to litmus. Milk was coagulated by acid production. Glucose, lactose and maltose were fermented, glycerin slightly and sucrose not at all, with production of acid and gas; the latter was found to be CO<sub>2</sub>, H<sub>2</sub>, CH<sub>4</sub> and a trace of N<sub>2</sub>. The organism was aerobic and facultative. It was decidedly pathogenic for mice and guinea pigs.

The organism seems to be a well characterized, distinct species, which should be recognized.

Migula (1900) reduced the name from trinomial form to *Bacterium chinense*. Bergy *et al* (1923) first placed the species in the genus *Aerobacter*. (Note: In the Manual of Determinative Bacteriology (1923) by Bergy *et al*, the name is incorrectly spelled "chiense".)

**Specific Diagnosis:** A non-motile rod, conforming to the generic diagnosis. Acid and gas are produced from glycerol, but not from sucrose. Gelatin is not liquefied. Litmus is slowly acidified and coagulated. Was first isolated from Chinese ink. Is pathogenic for white mice and guinea pigs.



4. *Aerobacter cloacae* (Jordan) Bergey *et al*, 1923.

Alternative: 15. *Bacterium cloacae* (Jordan) Lehm and Neum., 1896.

SYNONYMY: *Bacillus cloacae* Jordan, 1890; *Cloaca cloacae* (Jordan) Castellani and Chalmers, 1919.

Jordan (1890) isolated this organism from sewage at Lawrence, Mass. He described it as a short, plump, oval bacillus, with rounded ends, about  $0.8\text{--}1.9\mu$  long by  $0.7\text{--}1.0\mu$  broad, non-spore-forming, motile, aerobic and facultative. On gelatin, the deep colonies were round and yellowish; surface colonies slightly bluish with irregularly notched edges. Gelatin was liquefied quickly. In gelatin tubes the growth was rapid. Growth was good along the line of inoculation, a scum formed on the surface and a heavy, flocculent, whitish precipitate formed. On agar the growth was moist, slimy, porcelain white. On potato, yellowish-white, rapid growth. Milk was coagulated with a strong acid reaction. Bouillon was turbid with a slight scum and considerable whitish precipitate. Nitrates were reduced to nitrites.

Other investigators have added considerably to our knowledge of the organism. It has been found to produce indol from peptone, acetyl-methyl-carbinol from glucose and to ferment a number of carbohydrates with production of acid and gas ( $\text{CO}_2$  and  $\text{H}_2$ ).

**Specific Diagnosis:** Motile rods,  $0.5$  to  $1.0\mu$  broad by  $0.8$  to  $2.0\mu$  long, conforming to the generic diagnosis. Sucrose is fermented with acid and gas production; glycerol, starch, dulcitol and inositol are rarely attacked and adonitol is not fermented. Gelatin is usually liquefied. Indol is usually produced. Litmus milk is acidified and coagulated. Originally isolated from sewage. Found in the alimentary tract.

5. *Aerobacter levans* (Wolffin) Bergey *et al*, 1923.

Alternative: 16. *Bacterium levans* (Wolffin) Lehm - and Neum., 1896.

SYNONYMY: *Bacillus levans* Wolffin, 1894; *Cloaca levans* (Wolffin) Castellani and Chalmers, 1919.

This organism was isolated by Wolffin (1893), under the direction of Professor Lehmann, from sour dough. According to descriptions by Wolffin (1894) and by Lehmann (1894) the organism was identical with *Bacterium coli commune* in its morphological and cultural characters. It was aerobic and facultative, did not liquefy gelatin, did not coagulate milk, did not produce indol and fermented glucose with acid and gas ( $\text{H}_2$ :  $\text{CO}_2$ : 1:3). It also produced gas ( $\text{H}_2$  and  $\text{N}_2$ ) from sugar free bouillon. According to this description the organism could hardly be accepted in the colon-aerogenes section. However, later work by F. Frankel (1896) and by Papasoteriu (1901) with the same organism used by Wolffin and other strains isolated by themselves showed Wolffin to have been mistaken in some characters. They found indol to be produced and milk coagulated though sometimes only after 5-6 day incubation. Apparently, as Papasoteriu says, Wolffin recorded results after very short incubation. MacConkey (1906) quotes Hollinger (1902) as finding *B. levans* able to liquefy gelatin, the rate of liquefaction being very varied, sometimes taking 1-2 months. MacConkey (1906) himself studied *B. levans*, using a culture secured from Kral's laboratory, which we may assume was a descendant of the original. He found it to be motile, able to liquefy gelatin, not producing indol, Voges-Proskauer positive, producing acid and gas from glucose and lactose but



not from sucrose. In addition he gave its fermentation reaction on a long list of carbohydrates.

The species is considered adequately described, and the name valid, the accepted description being that given by Frankel and Papasoteriu and emended by MacConkey.

**Specific Diagnosis:** A motile rod,  $0.6\mu$  broad by  $1.8\mu$  long, conforming to the generic diagnosis. Sucrose, dulcitol, inositol and adonitol are not fermented. Indol is rarely produced. Gelatin is usually liquefied. Acid and curd are formed in litmus milk. Isolated from fermented dough. Found in soil and occasionally in the alimentary tract.

### Genus 3. *PROTEUS* Hauser, 1885

**SYNONYMY:** *Encapsulatus* (in part) Castellani and Chalmers, 1919; *Klebsiella* (in part) Trevisan, 1885; *Wesenbergus* (in part) Castellani and Chalmers, 1919.

*Proteus* was used by Hauser (1885) in a generic sense but without definition. Three organisms were described: *Proteus vulgaris*, *Proteus mirabilis* and *Proteus zenkeri*. The three agreed in being motile, non-spore-forming rods with great variability in morphology. They produced apparently motile "islands" on the moist surface of solid media. All were associated with putrefaction and decay. They were differentiated by Hauser on the basis of their relative abilities to attack gelatin.

Rogers, Clark and Lubs (1918) characterized the group as follows:

Short, thick, Gram-negative bacillus, tendency to ferment carbohydrates to some degree; normal habitat, intestines of warm blooded animals. Liquefies gelatin and under certain conditions forms characteristic "swarming" colonies. Acid and gas from glucose, but lactose is not fermented.

Jordan (1903) in describing the "Proteus type," adds to its characteristics the ability to ferment sucrose, but says lactose may be fermented, though rarely. He placed no emphasis upon morphological or cultural characters. In fact as Buchanan (1925) points out, few authors until recently have considered the pleomorphism of these forms as distinctive.

Buchanan (1918) used the name for a genus with the following description:

Short rods, showing great variation in morphology, filamentous and bent rods as involution forms frequent. Motile by means of peritrichous flagella. The species commonly produce motile "islands" on the surface of moist solid media. No spores. Gram-negative. Usually liquefying gelatin rapidly in absence of carbohydrates. Usually producing acid and gas from certain carbohydrates. In general the species are closely associated with decay and putrefaction, sometimes pathogenic.

The type species is *Proteus vulgaris* Hauser.

Werner and Rettger (1919) have made a careful study of the group. They concluded *Proteus zenkeri* of Hauser to be identical with *B. zopfii* for which they created a new genus *Zopfius*. All of their strains fermented glucose and sucrose with acid and gas production. Pure lactose was never fermented. Maltose fermentation was variable and was the only property they found which could be used for subdivision of the group into species.

Winslow *et al* (Committee, S. A. B., 1920) used *Proteus* with almost the same description as that of Buchanan for the first of two genera composing the tribe *Bacteraceae*. They added the statements that gelatin is liquefied,

and glucose and sucrose (but usually not lactose) are fermented with production of acid and gas (the latter being CO<sub>2</sub> only).

Weldin and Levine (1923) simply characterized their subgenus *Proteus* of the genus *Bacterium* by fermentation of glucose and sucrose, but not lactose, with formation of acid and gas.

The name has been used by Bergey *et al* (1923) with the following description:

Highly pleomorphic rods. Filamentous and curved rods are common as involution forms. Gram-negative. Actively motile, possessing peritrichous flagella. Produce characteristic amoeboid colonies on moist media and decompose proteins. Ferment dextrose and sucrose, but not lactose. Do not produce acetyl-methyl-carbinol.

The type species is *Proteus vulgaris* Hauser.

Little emphasis can be placed on morphological variations, especially since the work of Henrici and others on some of the organisms of the colon-typhoid series. Undoubtedly, involution forms are more easily demonstrated with these than with some of the other intestinal types. They possess peculiar cultural characters on certain media. These characteristics are comparative, however, and clear cut physiological characters would seem to be more reliable for use in differentiation. Their actions on the sugars, glucose, lactose and sucrose, constitute such characters.

Liquefaction of gelatin has been listed quite regularly as a characteristic of the *Proteus* species. Wenner and Rettger (1919) and others who have worked with such organisms point out that the property of liquefying gelatin was often lost by their cultures of *vulgaris* upon continued laboratory cultivation. In the literature many organisms are described which resemble *Proteus vulgaris* more clearly than they do any other type species, but which are gelatin non-liquefiers. In view of the instability of gelatin liquefaction this character is not included in the generic definition.

**Generic Diagnosis:** *Gram-negative, non-spore-forming rods usually highly pleomorphic in form. Usually motile. Produce gas from both glucose and sucrose, but not from lactose. Pathogenicity slight.*

Type species: *Proteus vulgaris* Hauser.

#### KEY TO SPECIES OF THE GENUS *PROTEUS*

- a. Neither acid nor gas formed from mannitol.
  - b. Acid and gas formed from maltose.
    1. *Proteus vulgaris*.
  - bb. Neither acid nor gas formed from maltose.
    2. *Proteus mirabilis*.
- aa. Acid and gas formed from mannitol.
  - b. Gelatin not liquefied.
    - c. Motile.
      - d. Acid and gas formed from salicin.
        3. *Proteus infantum*.
      - dd. Neither acid nor gas formed from salicin.
        4. *Proteus valeriei*.
    - cc. Non-motile.

- d. Capsules formed; indol not formed.
  - 5. *Proteus pneumoniae*.
- dd. Capsules not formed; indol formed.
  - 6. *Proteus asiaticus*.
  - 7. *Proteus hydrophilus*.
- bb. Gelatin liquefied.

SPECIES OF *PROTEUS*1. *Proteus vulgaris* Hauser, 1885.

Alternative: 17. *Bacterium vulgare* (Hauser) Lehm. and Neum. 1896.

SYNONYMY: *Bacillus proteus* Trevisan, 1889; *Bacillus Proteus vulgaris* (Hauser) Kruse in Flügge, 1896; *Bacterium vulgare* (Hauser) Chester, 1897; *Bacillus vulgaris* (Hauser) Migula, 1900; *Bacterium proteus anindologenes* von Loghem, 1918; *Proteus proteus vulgaris* (Hauser) Castellani and Chalmers, 1919.

Hauser (1885) described this organism as one of three species of a new genus *Proteus*. In his description he stressed particularly the variability of form which these organisms may assume and the amoeboid wandering colonies. He separated his three species on the basis of their relative ability to liquefy gelatin, *Proteus vulgaris* liquefying it rapidly, *P. mirabilis* slowly and *P. zenkeri* not at all. Later he decided that the last two species might be only varieties of *Proteus vulgaris*.

Early systematists accepted *Proteus vulgaris* with gradual elaboration of its description. It was soon found to be able to ferment glucose and sucrose with acid and gas production, but not lactose. The most exhaustive study of the group has been made by Wenner and Rettger (1919). These authors worked with 84 strains of *Proteus* group organisms, 58 of which were secured from other laboratories. From these and from their studies of the literature, they concluded that *Proteus vulgaris* and *Proteus mirabilis* were identical, while *Proteus zenkeri* resembled an organism previously described by Kurth (*Bacterium zopfii*). The property of liquefying gelatin they found to be too irregular and inconstant to serve as a basis for separation of species. They placed *Proteus zenkeri* in a new genus *Zopfius*. With regard to *P. vulgaris* and *P. mirabilis* they made the following suggestion:

"The *Proteus* genus comprises a large group of organisms which can be subdivided on the basis of their action on maltose into two distinct species. For the species fermenting this sugar the name *Proteus vulgaris* is suggested, and for the species failing to attack it, the name *Proteus mirabilis*. By retaining these names the nomenclature would be simplified. The differentiating characters of Hauser must be set aside, however, in order to avoid confusion."<sup>10</sup>

Their suggestion for differentiation of the two species on the basis of maltose fermentation was followed by Weldin and Levine (1923) and by Bergey *et al* (1923). It seems to be a satisfactory and convenient procedure and is here accepted.

\* The character referred to was rate of gelatin liquefaction.

The strains X2 and X19, used in the Weil-Felix reaction, agree, according to the best information at hand, culturally and morphologically, with *Proteus vulgaris*, but differ from it in their serological reactions. They should probably be considered varieties of the latter.

**Specific Diagnosis:** *A motile rod, conforming to the generic diagnosis. Sucrose fermented rapidly with acid and gas production; maltose fermented with acid and gas; mannitol and dextrin not fermented. Gelatin liquefied, at least by freshly isolated cultures. Indol is produced. Found in putrefying materials.*

2. *Proteus mirabilis* Hauser, 1885.

Alternative: 18. *Bacterium mirabile* (Hauser) Chester, 1897.

SYNONYMY: *Bacillus mirabilis* Trevisan, 1889; *Bacillus proteus mirabilis* (Hauser) Kruse, 1896.

This was one of three species first described by Hauser (1885). It was isolated by him from putrefying animal matter. For discussion, see *Proteus vulgaris*.

**Specific Diagnosis:** *A motile rod, conforming to the generic diagnosis. Sucrose slowly fermented with acid and gas production; maltose, mannitol and dextrin not fermented. Gelatin liquefied, at least when freshly isolated. Found in putrefying materials.*

3. *Proteus infantum* (Weldin and Levine) Comb. nov.

Alternative: 19. *Bacterium infantum* Weldin and Levine, 1923.

SYNONYMY: Brisbane organism, Dean, 1920.

This organism was first isolated by Dean (1920) from the urine and faeces of a child. Later it was secured from a number of faeces samples sent to him for examination for dysentery bacilli. He found it to be a motile rod, not able to liquefy gelatin, producing indol and producing acid and gas from glucose and sucrose, but not from lactose. In litmus milk it produced acid on the fifth day, clotted milk on the tenth and cleared it on the fifteenth day. In its agglutination reaction it appeared to be more closely linked to the paratyphoid B organism than to the paratyphoid A or typhoid organisms.

Weldin and Levine (1923) concluded that this organism was sufficiently described for recognition. They, accordingly, gave it the name *Bacterium infantum* and placed it in their subgenus *Proteus*, where its fermentation reactions clearly showed it to belong.

**Specific Diagnosis:** *A motile rod, conforming to the generic diagnosis. Ferments mannitol, maltose, salicin, raffinose and arabinose with acid and gas production, but does not attack inulin nor adonitol. Gelatin is not liquefied. Found in human urine and faeces.*

4. *Proteus valeriei* (Weldin and Levine) Bergey *et al*, 1923.

Alternative: 20. *Bacterium valeriei* Weldin and Levine, 1923.

SYNONYMY: Valerie 21, Boycott, 1906; *Bacillus asiaticus mobilis* Castellani, 1916; *Salmonella asiaticus mobilis* (Cast.) Castellani and Chalmers, 1919.

This organism was isolated by Boycott (1906) from the stool of a patient and described under the term "Valerie 21." It was actively motile,



did not liquefy gelatin, produced indol and acidified and clotted milk. It produced acid and gas from glucose, maltose, sucrose and mannitol as well as a number of other carbohydrates, but had no apparent action on lactose or salicin. It was pathogenic for guinea pigs when injected intraperitoneally. Agglutinin-absorption tests with "Schottmüller B" (*Salmonella schottmülleri*) and the organism of Brion and Kayser (*Salmonella paratyphi*) showed no relation to these two organisms.

Castellani (1916) described an organism found in the Adriatic-Balkan zone, which had almost identical morphological and physiological characters. He called it *Bacillus asiaticus mobilis*.

Weldin and Levine (1923) used the name *Bacterium valeriei* for Boycott's organism.

The organism was adequately described. Its ability to ferment glucose and sucrose, but not lactose, places it in the *Proteus* group.

The name *Bacillus asiaticus mobilis*, being a trinomial, must be rejected; *Proteus asiaticus* and *Bacterium asiaticum* have already been used for another recognized species (see *Proteus asiaticus*), and *Proteus mobilis* is a type of name which should be avoided as expressing a character common to all or nearly all members of the group (Recommendation XIV of the Botanical Code). *Proteus valeriei* is thus considered a valid name for the species.

**Specific Diagnosis:** A motile rod, conforming to the generic diagnosis. Produces acid and gas from mannitol, maltose, raffinose and arabinose, but not from salicin nor inulin. Gelatin is not liquefied. Found in the intestines of man.

##### 5. *Proteus pneumoniae* (Zopf) Comb. nov.

Alternative: 21. *Bacterium friedlander* (Trevisan) Comb. nov.

**SYNONYMY:** *Pneumonie Coccus*, Friedlander, 1883; *Bacterium pneumoniae crouposae* Zopf, 1885; *Bacillus Pneumoniae* (Friedlander) Flüge, 1886; *Klebsiella pneumoniae* Trevisan, 1887; *Bacillus capsulatus pneumonicus* Banti, 1888; *Klebsiella friedlander* Trevisan, 1889; *Pneumobacillus* Eisenberg, 1891; *Bacterium pneumonicum* (Friedlander) Migula, 1895; *Bacterium pneumoniae* Friedlander, Lehmann and Neumann, 1896; *Bacillus Friedländer* Buchan, 1910.

This organism was first described but not named by Friedlander in 1882. He isolated it from the pleuritic and pericardial exudate from a case of acute pneumonia. It was described as a coccus, though he stated that the organisms were usually ellipsoid in shape, their length being about one micron and their breadth a little less. It was usually in pairs, though sometimes in short chains. In a later publication (1883) he described capsule formation; these capsules were always observed with organisms from the animal body, but not in cultures on gelatin or blood serum.

Later investigators working with the same organism have shown it to be unquestionably a rod, Gram-negative, with cultural characters typical of the colon-typoid bacilli, fermenting a number of carbohydrates, including glucose and sucrose with acid and gas ( $\text{CO}_2$  and  $\text{H}_2$ ).

There is considerable disagreement among various investigators as to the ability of this organism to ferment lactose. Grimbert (1895) stated that it was able to ferment this sugar with acid production. Later (1896) he added gas production from lactose. Nicolle and Hébert (1897) con-



firmed Grimbert's findings. Lehmann and Neumann (1901) stated that it produced abundant acid, together with  $\text{CO}_2$  and  $\text{H}_2$  from grape- and milk-sugar. Strong (1889) working with cultures from Kral's laboratory, from the Göttingen Institute and from his own isolations, found no acid and "wenig oder gar kein Gaz bei Milchzucker gebildet." Perkins (1904) worked with a subculture from Kral's laboratory and found it produced neither acid nor gas from lactose. MacConkey (1905) also working with a culture obtained indirectly from Kral, records acid, but not gas, from lactose. Another culture obtained from H. Spitta did produce gas as well as acid. Coulter (1917) studied eleven strains of Friedlander's bacillus isolated by himself, and found none of them able to ferment lactose. It is quite evident that the investigators who were supposedly working with lineal descendants of the original strain found it incapable of producing gas from lactose. The species is, therefore, considered in the subgenus *Proteus* because it produces gas from glucose and sucrose, but not from lactose.

Zopf (1885) used the name *Bacterium pneumoniae crouposae* for the Friedlander bacillus. This name, being a trinomial, cannot stand in its entirety. Flügge (1886) used the name *Bacillus pneumoniae* for the species. The combination *Bacterium pneumoniae* is invalid for this organism, since this specific designation was first used by Migula (1895) for the pneumonia organism of Weichselbaum now generally recognized under the name *Diplococcus pneumoniae* as the type species for the genus *Diplococcus*. Trevisan (1889) used the term *Klebsiella Friedländeri* for this species; *Friedlanderi* may, therefore, be considered the valid specific name for the organism when the generic term *Bacterium* is used.

However, the combination *Proteus pneumoniae* has not been used previously, and since *pneumoniae* has priority over *friedlanderi*, this must be the accepted name when the organism is considered to be in the genus *Proteus*.

**Specific Diagnosis:** A non-motile rod, conforming to the generic diagnosis. Maltose, mannitol, arabinose, raffinose and dulcitol are fermented with acid and gas production; inulin is not attacked. Gelatin is not liquefied. Indol is not formed. Capsules are generally produced when the organism is grown in milk. Found in saliva and exudates. Associated with pneumonia, bronchitis and various inflammations of the respiratory tract.

6. *Proteus asiaticus* (Castellani) Bergey *et al.*, 1923.

Alternative: 22. *Bacterium asiaticum* (Castellani) Weldin and Levine, 1923.

**SYNONYMY:** *Bacillus asiaticus* (No. 1 and No. 2) Castellani, 1912; *Salmonella asiaticus* (Cast.) Castellani and Chalmers, 1919; *Bacterium* (*Proteus*) *asiaticum* Weldin and Levine, 1923.

This organism was isolated by Castellani (1912) from the blood and stools of a patient suffering from a long protracted fever following ankylostomiasis. Serum reactions and the results of vaccination indicated a probable causal relationship of the organism to the fever. It was described as a short, rod-like organism,  $2-5\mu$  in length, non-motile, Gram-negative. Growth on agar was typhoid-like; in broth there was general turbidity and pellicle formation. Gelatin was not liquefied, indol production was slight,

litmus milk was acid, with reversion to alkalinity. A number of carbohydrates were attacked, including glucose, sucrose and mannitol. Lactose was not fermented. Two varieties are described, the differences largely being intensity and rate of some of the reactions. They were combined as one species in later descriptions (1916, 1919).

The species is adequately described and sufficiently distinct to warrant recognition.

**Specific Diagnosis:** A rod, 2 to 5 $\mu$  in length, conforming to the generic diagnosis. Non-motile. Ferments mannitol, maltose, sorbitol and glycerol, with acid and gas production; does not attack inulin nor adonitol. Gelatin is not liquefied. Indol is produced. Litmus milk becomes acid followed by alkalinity; marked capsule formation does not occur. Found in the intestinal tract of man.

7. *Proteus hydrophilus* (Sanarelli) Bergey et al, 1923.

Alternative: 23. *Bacterium hydrophilum* (Sanarelli) Chester, 1897.

SYNONYMY: *Bacillus hydrophilus fuscus* Sanarelli, 1891; *Bacterium hydrophilus fuscus* (Sanarelli) Chester, 1897; *Bacillus hydrophilus* (Sanarelli) Chester, 1901; *Bacillus ranicida* (?) Ernst, 1890; *Bacterium ranicida* (?) (Ernst) Lehmann and Neumann, 1901.

Sanarelli (1891) obtained this organism from the lymph of frogs suffering from a fatal infectious disease. It was a Gram-negative short rod, sometimes growing into long filaments, aerobic, gelatin-liquefying and motile. On glycerin agar at 37° C. it grew luxuriantly, soon covering the entire surface and exhibiting a slight fluorescence which soon disappeared. Blood serum was liquefied. On potato it developed a yellowish to brownish growth. It was pathogenic not only for "cold-blooded" animals, but also for guinea pigs, rabbits, dogs, cats, mice, chickens and pigeons. Sanarelli called it *Bacillus hydrophilus fuscus*.

A bacillus was carefully studied and described in detail by Emerson and Norris (1905), which corresponded morphologically and culturally to Sanarelli's description, but differed slightly as to pathogenicity. They found guinea pigs susceptible, but not rabbits; no other animals were tested. In addition they described it as highly pleomorphic. Milk was acidified, coagulated and peptonized. Dextrose, sucrose and mannitol were fermented with acid and gas production, but gas was never formed from lactose. The ability to produce gas from sugars was reported lost with continued cultivation. Nitrates were reduced to nitrites and indol was slowly produced. It produced a slight yellowish pigment on some media; however, "pigment formation was so variable that no constant factors controlling its occurrence were determinable." The authors concluded that their organism was identical with that of Sanarelli.

Ernst in 1890 described an organism under the name *Bacillus ranicida* which corresponds very closely in its characters to *Bacillus hydrophilus fuscus* Sanarelli, and there may be some question as to whether his name should not be used in place of Sanarelli's. However, both his and Sanarelli's descriptions were incomplete and inadequate for recognition of the species were it not for the work of Emerson and Norris. These authors definitely stated that their organism corresponded more closely to *Bacillus hydrophilus fuscus* Sanarelli than to *Bacillus ranicida* Ernst. In view of

this statement it seems best to retain the specific name *hydrophilus* and consider *B. ranicida* a possible synonym.

Its characters place it definitely in the genus *Proteus*. Chester (1901) reduced the name to binomial form (*Bacillus hydrophilus*) and Weldin and Levine (1923) placed it in their subgenus *Proteus* of the genus *Bacterium*.

**Specific Diagnosis:** A motile rod, conforming to the generic diagnosis. Mannitol and maltose are fermented with acid and gas production. Gelatin is liquefied. Found in the lymph of frogs dead from a disease known as "red leg." Pathogenic for frogs, salamanders, fish, guinea pigs.

Genus 4. *SALMONELLA* Lignières, 1909.

**SYNONYMY:** Includes *Balkanella* Castellani and Chalmers, 1919; *Wesenbergus* (in part) Castellani and Chalmers, 1919.

This name was first proposed by Lignières (1900) for a genus to include the hog cholera organism of Salmon. He stated: "Le microbe de la Schweineseptikämie rentre dans le groupe des *Pasteurella*; celui du Hog-cholera ou Schweinepest est très différent, il pourrait servir de prototype pour la création d'un autre groupe, celui des *Salmonella*."

Buchanan (1918) used the name for a subgenus of the genus *Bacterium* with the following description:

Fermenting glucose but not lactose with formation of acid and gas. Frequently pathogenic. Type species: *Bacterium (Salmonella) cholerae suis*.

Castellani and Chalmers (1919) recognized it as the name of a genus of the tribe *Ebertheae*. While they included the ability to ferment mannitol (at least partially) in their characterization, they divided the genus into three groups, one of them being the Morgan group which does not attack mannitol at all.

Weldin and Levine (1923) used the name for a subgenus. They described it as including members of the genus *Bacterium* which ferment glucose, but neither lactose nor sucrose with acid and gas.

Bergey *et al* (1923) also recognized *Salmonella* as a genus. In their key to genera of the tribe *Bactereae*, it is separated from the other genera of the colon-typhoid series by ability to produce acid and gas from glucose, and inability to attack lactose and sucrose. Their description reads as follows:

Motile forms occurring in the intestinal canal of animals, in various types of acute, inflammatory conditions. Attack numerous carbohydrates with the formation of both acid and gas. In general do not form acetyl-methyl-carbinol. Type species: *Salmonella schottmülleri*.

Their type species is evidently in error. In the 1925 edition it is changed to *Salmonella suipestifer* (Kruse) Lignières.

Duncan (1924) would restrict the group to exclude the Morgan group of bacilli, *Bact. paratyphi*, and a number of other species commonly included by recent authors. He suggests the following characterization for *Salmonella*:

Gram-negative non-sporing bacilli, usually actively motile, which do not ferment lactose, saccharose or salicin, do not liquefy gelatin, and never give the indol reaction. In litmus-milk they cause a transient acidity followed after 48 hours by alkalinity. They ferment glucose, mannitol and maltose with production of acid and gas.

Such a diagnosis limits the species to be included in the genus to those which conform very closely to the type, and excludes others, which, on the basis of their serological as well as physiological reactions, are clearly related to species which Duncan would include.

The best method at present seems to be to use the term *Salmonella* for that part of the colon-typhoid series intermediate between the colon-aerogenes-proteus types and the typhoid-dysentery types. Most of this group are slightly pathogenic and many are related serologically. If further subdivision is thought necessary it might be indicated by sections such as the Gärtner section, (mannitol, acid and gas; xylose, acid and gas), the paratyphi section, (mannitol, acid and gas; xylose, negative), and the Morgan section (mannitol, negative).

**Generic Diagnosis:** *Gram-negative, non-spore-forming rods. Usually motile. Produce gas from glucose, but not from lactose nor sucrose. Frequently pathogenic.*

**Type species:** *Salmonella cholerae suis* (Smith, Th., 1894).

#### KEY TO SPECIES OF THE GENUS *SALMONELLA*.

- a. Acetyl-methyl-carbinol not produced from glucose.
  - b. Acid produced from mannitol.
    - c. Acid and gas produced from maltose.
      - d. Acid and gas produced from xylose; litmus milk, slightly acid, reverting rapidly to an alkaline reaction.
        - e. Dulcitol and arabinose not fermented or fermented very slowly with acid and gas production.
          - f. Neither acid nor gas produced from dextrin; agglutinins from *Sal. cholerae suis* serum completely absorbed; those from *Sal. icteroides* not completely absorbed.
            1. *Salmonella cholerae suis*.
          - ff. Acid and gas produced from dextrin; agglutinins from *Sal. cholerae suis* serum not completely absorbed; those from *Sal. icteroides* completely absorbed.
            2. *Salmonella icteroides*.
    - ee. Dulcitol and arabinose rapidly fermented with acid and gas production.
      - f. Acid and gas produced from inositol.
        - g. Gas produced from mannitol; salicin not fermented; raffinose usually not fermented.
          - h. Acid and gas produced from raffinose.
            3. *Salmonella psittacosis*.
          - hh. Neither acid nor gas produced from raffinose.
            - i. Agglutinins from *Sal. schottmülleri* serum completely absorbed.
              4. *Salmonella schottmülleri*.

- ii. Agglutinins from *Sal. schottmülleri* serum not completely absorbed.
  - j. Agglutinins from *Sal. aertrycke* serum completely absorbed; those from *Sal. anatum* not completely absorbed.
    - 5. *Salmonella aertrycke*.
  - jj. Agglutinins from *Sal. aertrycke* serum not completely absorbed; those from *Sal. anatum* completely absorbed.
    - 6. *Salmonella anatum*.
- gg. Acid, not gas, produced from mannitol; salicin usually, raffinose always fermented with acid and gas production.
  - 7. *Salmonella veboda*.
- ff. Neither acid nor gas produced from inositol.
  - g. Acid and gas produced from salicin; indol produced.
    - 8. *Salmonella columbensis*.
  - gg. Neither acid nor gas produced from salicin; indol rarely produced.
    - h. Not agglutinated by *Sal. enteritidis* serum.
      - 9. *Salmonella hirschfeldii*.
    - hh. Agglutinated by *Sal. enteritidis* serum.
      - i. Agglutinins from *Sal. enteritidis* serum completely absorbed; those from *Sal. abortivo-equinum* not completely absorbed.
        - 10. *Salmonella enteritidis*.
      - ii. Agglutinins from *Sal. enteritidis* serum not completely absorbed; those from *Sal. abortivo-equinum* completely absorbed.
        - 11. *Salmonella abortivo-equinum*.
  - dd. Neither acid nor gas produced from xylose; litmus milk, slightly acid, reverting very slowly if at all.
    - e. Neither acid nor gas produced from inositol and raffinose; indol not produced.
      - f. Acid and gas produced from levulose and arabinose.
        - 12. *Salmonella paratyphi*.
    - ff. Neither acid nor gas produced from levulose and arabinose.
      - 13. *Salmonella woliniæ*.



ee. Acid and gas produced from inositol and raffinose; indol produced.

14. *Salmonella watareka*.

cc. Neither acid nor gas produced from maltose.

15. *Salmonella pullorum*.

bb. Neither acid nor gas produced from mannitol.

c. Neither acid nor gas produced from maltose and dextrin.

d. Acid and gas produced from levulose.

16. *Salmonella morganii*.

dd. Neither acid nor gas produced from levulose.

17. *Salmonella foetida*.

cc. Acid and gas produced from maltose and dextrin.

d. Non-motile; acid and gas produced from salicin and sorbitol.

18. *Salmonella giunai*.

dd. Motile; neither acid nor gas produced from salicin and sorbitol.

19. *Salmonella macfadyeanii*.

aa. Acetyl-methyl-carbinol produced from glucose.

20. *Salmonella archibaldii*.

#### SPECIES OF *SALMONELLA*

1. *Salmonella cholerae suis* (Smith, Th.) Comb. Nov.

Alternative: 24. *Bacterium cholerae suis* (Th. Smith) Holland, 1920.

SYNONYMY: *Bacillus cholerae suis* Smith, Th., 1894; *Bacillus cholerae suum*, Migula, 1895; *Bacillus suipestifer* Kruse, 1896; *Bacterium suipestifer* Chester, 1897; *Bacterium cholerae suum* (Migula) Lehmann and Neumann, 1896; *Bacillus salmoni* (Trevisan) Chester, 1901; *Bacterium intestinale suis* LeBlaye and Guggenheim, 1914; *Bacillus suis* Krumwiede, Kohn, and Valentine, 1918; *Salmonella suipestifer* (Kruse) Castellani and Chalmers, 1919; not *Pasteurella salmoni* Trevisan, 1889.

Salmon and Smith (1885) in a study of disease of hogs which they called American swine plague, isolated an organism and described it as the "Bacterium of swine plague." The organisms appeared as elongated ovals, usually in pairs. When stained with methyl violet, many bacilli presented a center paler than the periphery. They stated further, "The darker portion is not localized at two extremities, as in the bacteria of septicemia in rabbits." The bacillus was motile and did not liquefy gelatine. It grew well on potato, blood serum, and in milk. Appearance of milk was not changed. Spores were not found. It was pathogenic for laboratory animals.

In 1886 these authors published additional information on the organism. In the meantime, it had become evident that there were two distinct infectious diseases of hogs, both of which had previously been known as

swine plague. Salmon and Smith called these two diseases swine plague and hog cholera, using the latter for the disease described in 1885, and the term, "hog cholera bacillus," for the organism previously called by them the "swine plague bacillus." The disease which they now (1886) called swine plague, had also been shown to exist in Europe, where it was likewise called swine plague or "Schweineseuche".

In 1894, Smith again emphasized that his "hog cholera bacillus" 1886, was identical with his "swine plague bacillus" 1885, and that the name "swine plague bacterium" was now being used for an organism like one similarly designated in Europe. At this time he gave the name *Bacillus cholerae suis* to the "hog cholera" bacterium. To the previous descriptions, he added that acid and gas ( $\text{CO}_2$  and  $\text{H}_2$ ) were evolved from glucose, but that lactose and sucrose were not attacked.

Kruse (1896) referring to an organism which he said was first isolated by Salmon and Smith (1885) and called by them the "Hog-cholera-Bacillus," created the name *Bacillus suipestifer*. Most of the authors who have worked on the organisms of the intermediate group have accepted Kruse's name *suipestifer*; a few have used *cholerae suis*, which evidently has priority and is the valid name. The writer has been informed of some experimental work, the results of which have not yet been made available, which indicate very strongly that there are distinct differences between American strains designated as *cholerae suis* and European cultures supposedly *suipestifer*. There is a possibility that each name should be recognized as representing distinct species.

The authors who have worked with the organisms of the intermediate group have generally recognized the species either under the name *suipestifer* or as *cholerae suis*. Ford (1905), Harding and Ostenberg (1912) and Krumwiede, Pratt and Kohn (1916) have shown it to be a xylose fermenter, thereby separating it from *Sal. paratyphi* (para A). The agglutination test was used for some time to differentiate the *hog cholera* bacillus from Gärtner's organisms. Differences in ability to attack arabinose and dulcitol were noted by Ford (1905) and Ditthorn (1913). Jordan (1917) characterized *cholerae suis* strains as fermenting arabinose and dulcitol slowly or not at all, while *schottmülleri* strains (para B) typically produced gas from these carbohydrates within 24 hours.

**Specific Diagnosis:** A motile rod, conforming to the generic diagnosis. It ferments maltose, mannitol and xylose with acid and gas production; dulcitol and arabinose are fermented very slowly if at all; adonitol, salicin, raffinose and dextrin are not attacked. Acetyl-methyl-carbinol is not formed from glucose. Litmus milk becomes acid, reverting rapidly to an alkaline reaction. Found in the intestines of hogs and as a secondary invader of tissues in hog cholera.

## 2. *Salmonella icteroides* (Sanarelli) Bergey et al, 1923.

Alternative: 25. *Bacterium icteroides* (Sanarelli) Lehmann and Neumann, 1901.

SYNONYMY: Bacille icétoïde Sanarelli, 1897; *Bacillus icteroides* (Sanarelli) Reed and Carroll, 1900.

This organism was first described by Sanarelli (1897), who isolated it from yellow fever cadavers and believed it to be the cause of the disease. He called it bacille icétoïde. His description of its growth on ordinary

laboratory media was quite detailed, but many of the peculiarities ascribed to it were shown, by Agramonte and others, not to be constant. It fermented glucose, but not lactose nor sucrose. Sanarelli's belief that it was the etiological factor in the disease yellow fever was soon shown to be incorrect. Reed and Carroll (1900) proved its close relationship to the hog cholera bacillus.

MacConkey (1905) secured a culture of the organism from Sanarelli and determined its fermentation reactions in a number of carbohydrates. Subsequent authors have added somewhat to the list of sugars attacked, the net results showing, however, that the organism is practically indistinguishable from *Sal. cholerae suis* on the basis of its physiological reactions. Reed and Carroll (1900) found the two organisms agglutinatively identical. Krumwiede, Kohn, and Valentine (1918), however, were able to differentiate the two species by use of the agglutinin-absorption test.

**Specific Diagnosis:** A motile rod, conforming to the generic diagnosis. Its cultural and physiological characters are the same as *Sal. cholerae suis*, except that dextrin and often raffinose are fermented with acid and gas production. It may be differentiated serologically from *Sal. cholerae suis* by the agglutinin-absorption test. It was originally isolated from yellow fever cadavers, but has been found to have no etiological relationship to the disease.

3. *Salmonella psittacosis* (Nocard) Castellani and Chalmers, 1919.

Alternative: 26. *Bacterium psittacosis* (Nocard) LeBlaye and Gugenheim, 1914.

SYNONYMY: *Bacillus psittacosis* Nocard, 1893.

*Bacillus psittacosis* was isolated by Nocard (1893) from the wings of parrots which had died from pneumonia. The organism was also isolated from the blood of humans who had become infected. Its morphological and cultural characters were those of the intermediate group of the colon-typhoid series.

MacConkey (1905) secured a culture from the Pasteur Institute (presumably a sub-culture of the original) and studied its action on a number of carbohydrates. He described it as producing acid and gas from glucose, maltose, arabinose, raffinose, mannitol, sorbitol, dulcitol and dextrin, but not from lactose or sucrose. Castellani and Chalmers (1910) and Castellani (1912) agreed with MacConkey as to the carbohydrates attacked and noted further, failure to attack inulin, salicin and adonitol, and reversion from an acid to an alkaline reaction in litmus milk. Castellani (1917) quoted Bainbridge (no date given) as considering *Sal. psittacosis* identical with *Sal. aertrycke*. Up to the present the writer has not succeeded in verifying this statement.

Perry (1920) isolated from a diseased parrot an organism which was identical with Nocard's bacillus. He tested it and a subculture of Nocard's original strain secured from Dr. Besredka, by agglutinin and agglutinin-absorption tests with strains of the paratyphoid B bacillus and *Sal. aertrycke*, and found them serologically identical with the latter. Apparently the organism is very closely related to *Sal. aertrycke*, the only difference noted being its ability to attack raffinose and dextrin and its specific pathogenicity.

**Specific Diagnosis:** A motile rod, conforming to the generic diagnosis. It ferments levulose, maltose, raffinose, arabinose, dextrin, mannitol and dulcitol with acid and gas production; adonitol and salicin are not attacked. Acetyl-methyl-carbinol is not formed from glucose. Litmus milk becomes acid, rapidly reverting to alkalinity. Serologically identical with *Salmonella aertrycke*. The cause of a pneumonia of parrots; also affecting man associated with sick parrots.

4. *Salmonella schottmülleri* (Winslow, Kligler and Rothberg) Bergey *et al.*, 1923.

Alternative: 27. *Bacterium schottmülleri* (Winslow, Kligler and Rothberg), Holland, 1920.

**SYNONYMY:** *Bacterium paratyphi* type B, Kayser, 1904; "Bacillus paratyphoid B, Schottmüller" Morgan, 1905; *Bacillus paratyphosus* B, Wilson, 1908; *Bacterium paratyphosum* B (Schottmüller) LeBlaye and Guggenheim, 1914; *Bacillus schottmülleri* Winslow, Kligler, Rothberg, 1919; *Salmonella paratyphosus* B (Schottmüller) Castellani and Chalmers, 1919.

Achard and Bensaude (1896) described organisms similar to the psittacosis organism of Nocard, which produced gas from glucose, maltose and mannitol, but not from lactose. Milk was not coagulated. They agglutinated weakly with typhoid serum. This latter test suggests the paratyphoid B rather than the A type. The authors designated them as "Bacilles paratyphiques."

Schottmüller (1900) likewise isolated organisms intermediate in character from the typhoid bacillus and *Bacterium coli*. Kayser (1902, 1904) studied these organisms, along with a similar strain isolated by Brion and Kayser (1902), and concluded that there were two distinct species which he designated as *Bacterium paratyphi* Type A and *Bacterium paratyphi* Type B, the type A including Brion and Kayser's strain, and two of Schottmüller's strains, while type B included the rest of Schottmüller's strains (from 5 cases), as well as Achard and Bensaude's organism. (See *Salmonella paratyphi*.) The type B organisms differed agglutinatively from the type A, and in their ability to revert the reaction of litmus milk from acidity to alkalinity in two weeks time.

The organism (type B) has been isolated and studied in detail by subsequent investigators, until, at the present time, it may be clearly differentiated from related species on the basis of its cultural and serological reactions. Ford (1905) found it able to ferment xylose with acid and gas, which, as Krumwiede, Pratt and Kohn (1916) pointed out, offered a convenient method of differentiating it from paratyphoid A, which fails to attack this carbohydrate. Jordan (1917) used its ability to ferment rapidly arabinose and dulcitol to separate it from *Salmonella cholerae suis* and Weiss and Rice (1917) differentiated it from *Salmonella enteritidis* on its inositol fermentation.

The species has been commonly designated by the terms *B. paratyphoid* B and *Bacillus paratyphosus* B. Since neither of these names were in accepted form and are misleading as to the true biological relationships of the organism, Winslow, Kligler and Rothberg (1919) proposed the name *Bacillus schottmülleri* for the species.

Holland (1920) placed the species in the genus *Bacterium*, erroneously giving credit for the specific term to Winslow-Rottenberg-Parsons.



**Specific Diagnosis:** A motile rod, conforming to the generic diagnosis. It produces acid and gas from levulose, maltose, arabinose, xylose, dulcitol, mannitol, and inositol; but does not attack adonitol or salicin, and usually not raffinose. Acetyl-methyl-carbinol is not formed from glucose. Litmus milk becomes acid, reverting to alkalinity. Lead acetate medium is blackened. Is found in the human intestines and urine and as the causative agent of paratyphoid fever and food poisoning by use of meat from infected animals.

5. *Salmonella aertrycke* (DeNobele) Castellani and Chalmers, 1919.

Alternative: 28. *Bacterium aertrycke* (DeNobele) Weldin and Levine, 1923.

**SYNONYMY:** *Bacillus aertrycke* DeNobele, 1889, 1901; *Bacillus para-aertrycke* Castellani, 1916; *Salmonella para-aertrycke* (Cast.) Castellani and Chalmers, 1919.

This organism was secured by DeNobele (1889) from an outbreak of food-poisoning. In its morphological and cultural characters it was found to be similar to the Gärtner bacillus, the paratyphoid B. organism and to *Sal. cholerae suis*. It was found to be easily differentiated from *Sal. enteritidis* by the agglutination test. Bainbridge (1909) separated it from the paratyphoid B bacillus (*Sal. schottmülleri*) by means of the agglutinin-absorption test and, apparently, by the same test showed it to be identical with *Sal. cholerae suis*. The majority of investigators since Bainbridge's work have considered *aertrycke* indistinguishable from *cholerae suis*. However, Jordan (1917) found *aertrycke* strains, like *Sal. schottmülleri*, able to ferment dulcitol and arabinose rapidly, while *Sal. cholerae suis* fermented these carbohydrates slowly or not at all. The organisms may at present be differentiated from the other members of the intermediate group except *Sal. cholerae suis* by agglutination or by agglutinin-absorption tests, and from *Sal. cholerae suis* by carbohydrate fermentations, and is considered a distinct and valid species.

**Specific Diagnosis:** A motile rod, conforming to the generic diagnosis. Culturally, it can not be distinguished from *Sal. schottmülleri*, but may be differentiated from the latter by the agglutinin-absorption test. Dextrin is not fermented. The organism has been found in the intestinal tract of man and animals. It is sometimes associated with fevers of the paratyphoid type, and with cases of meat poisoning.

6. *Salmonella anatum* (Rettger and Scoville) Bergey et al, 1925.

Alternative: 29. *Bacterium anatum* Rettger and Scoville, 1920.

**SYNONYMY:** *Bacterium anatis* Rettger and Scoville, 1919; *Escherichia anata* (Rettger) Bergey et al, 1923.

This organism was first described by Rettger and Scoville (1919) under the name of *Bacterium anatis*. This name had to be discarded because it had been previously used by Cornil and Toupet (1888) for an organism resembling or identical with *Pasteurella aviseptica*. Accordingly, they changed it (1920) to *Bacterium anatum*. The organism was isolated from an intestinal disease of ducklings known as "keel". Its morphological and cultural characters showed it to be a member of the colon-typhoid series. Litmus milk was made acid and then alkaline. A number of carbohydrates were fermented with acid and gas production, including glucose, dextrin,



arabinose, dulcitol, inositol, xylose and mannitol. Lactose, sucrose, inulin, raffinose, adonitol and salicin were not fermented. The organism is evidently a member of the *Salmonella* group—closely resembling *Sal. schottmülleri*. Its agglutination reactions likewise link it to this bacillus. Work done at this laboratory (results not yet published) show that the two organisms may be differentiated by means of the agglutinin-absorption test.

**Specific Diagnosis:** A motile rod, conforming to the generic diagnosis, indistinguishable on the basis of morphological and cultural test from *Sal. schottmülleri* but differing from the latter serologically. Dextrin is fermented with acid and gas production; raffinose is not attacked. The causative agent of a disease of ducklings, known as "keel".

7. *Salmonella veboda* (Castellani) Castellani and Chalmers, 1919.

Alternative: 30. *Bacterium veboda* (Castellani) Weldin and Levine, 1923.

**SYNONYMY:** *Bacillus veboda* Castellani, 1917; *Bacillus willegodai* Castellani, 1917; *Salmonella willegodai* (Cast.) Castellani and Chalmers, 1919.

Castellani (1917) characterized this organism as a motile rod, Gram-negative, not liquefying gelatine nor serum, indol negative, producing acid and gas from a number of carbohydrates, including glucose, maltose, dextrin, dulcitol, raffinose, arabinose, sorbitol and inositol. Acid but not gas was produced from mannitol and rhamnose. Lactose, sucrose, adonitol, inulin and salicin were not attacked. Castellani and Chalmers (1919) included it in their genus *Salmonella*.

Its partial fermentation of the alcohol mannitol sets it apart from the rest of the organisms of the intermediate group. On this basis, although Castellani is evidently the only author who has described such a species, it is deemed worthy of recognition. Weldin and Levine (1923) included it in their subgenus *Salmonella* of the genus *Bacterium*.

*Bacillus willegodai* of Castellani differs from *veboda* by its ability to produce acid and gas from salicin, and gas from rhamnose, its inability to produce gas from dulcitol and levulose, and by slight indol production. Until further instances of its isolation are recorded, it is thought best to regard it as a synonym of *Sal. veboda*.

**Specific Diagnosis:** A motile rod, conforming to the generic diagnosis. Mannitol is fermented with acid production, but no gas; acid and usually gas are produced from dulcitol; arabinose, raffinose, inositol, dextrin and usually salicin, fermented with acid and gas; adonitol is not attacked. Litmus milk is acidified, reverting to alkalinity. Found in the human intestines.

8. *Salmonella columbensis* (Castellani) Castellani and Chalmers, 1919.

Alternative: 31. *Bacterium columbensis* Castellani, 1905.

**SYNONYMY:** *Bacillus columbensis* Castellani, 1917.

Castellani first described this organism in 1905 under the name *Bacterium columbense*. Later he decided it was identical with *Sal. schottmülleri*, but upon subsequent isolations and more careful study, he found it distinctly different from the latter. He isolated it from cases clinically similar to typhoid, but of medium severity. It gave distinctly negative

tests with typhoid serum, paratyphoid A serum and paratyphoid B serum. Culturally it was like paratyphoid B. Ordinarily it did not ferment lactose, though sometimes it produced a very slight amount of acid and gas. It is probable that the lactose might have been partially inverted on these occasions. It produced acid and gas from glucose, maltose, dulcitol, mannitol, arabinose, sorbitol and salicin, but not from sucrose, raffinose, adonitol, inulin or inositol. Its ability to ferment salicin separates it from *Sal. enteritidis*, and its lack of ability to attack inositol, as well as its agglutination reactions separate it from *Sal. schottmülleri*. The organism is apparently a valid species.

**Specific Diagnosis:** A rod conforming to the generic diagnosis. Motility variable. Acid and gas produced from levulose, maltose, xylose, mannitol, dulcitol, arabinose, salicin and dextrin; raffinose, inositol and adonitol are not attacked. Indol is produced. Litmus milk becomes slightly acid, reverting to alkalinity. Acetyl-methyl-carbinol is not produced from glucose. Found in the faeces and urine, and sometimes in the blood of men suffering from a disease resembling typhoid.

9. *Salmonella hirschfeldii* Sp. nov.

Alternative: 32. *Bacterium hirschfeldii* Sp. nov.

**SYNONYMY:** Paratyphoid C, Hirschfeld, 1919; *Bacillus paratyphosus* C, Castellani and Chalmers, 1919.

Uhlenhuth and Hubner (1908) proposed the term "paratyphosus C" for a race of bacilli found by them in pigs. They stated that it was culturally indistinguishable from *Sal. cholerae suis*, but was agglutinatively distinct from the latter and from the Gärtner bacillus. However, Uhlenhuth and his school did not differentiate between *Sal. cholerae suis* and paratyphoid B. It is impossible to say with certainty whether Uhlenhuth and Hubner were using *cholerae suis* or para B strains for comparison and their organism must be discarded as inadequately characterized, although there is a possibility that it may have been the same as the organism subsequently described by Hirschfeld.

Hirschfeld (1919) isolated from cases of enteric fever in the Serbian army, an organism which he labelled "paratyphoid C". This organism he considered distinct from the known paratyphoid organisms he was isolating from cases of enterica. Dudgeon and Urquhart (1921) working with the original strain of Hirschfeld's bacillus and with a strain secured by them which was similar in all respects to that of Hirschfeld, found them to be culturally indistinguishable from para B. Agglutinatively, however, they were distinct from para B, para A, the Gärtner bacillus and the Aerttrycke bacillus, but serologically identical with *cholerae suis*. Mackie and Bowen (1919) and Andrewes and Neaves (1921) likewise stated that "paratyphosus C Hirschfeld" is not agglutinated by para B serum, but is serologically identical with *cholerae suis*.

Mackie and Bowen (1919), TenBroeck (1920), and Andrewes and Neave (1921) found para C distinctly separated from *cholerae suis* by its ability to ferment rapidly dulcitol and arabinose. Dudgeon and Urquhart (1921) also found differences in fermentability on these two carbohydrates for the two species. Andrewes and Neave (1921) as well as Weiss and

Rice (1917) found para C unable to attack inositol, thus furnishing a cultural method of differentiating it from para B.

The information at hand indicates that we have in *paratyphosus* C Hirschfeld, a distinct and recognizable species. Since the term "*paratyphosus* C" can hardly be considered in correct form, the name *Salmonella hirschfeldii* (or *Bacterium hirschfeldii*) is proposed in its place, the name to be applied to the organism originally isolated by Hirschfeld.

**Specific Diagnosis:** An organism conforming to the generic diagnosis, and very similar to *Salmonella enteritidis* in its cultural and physiological characters. Levulose, maltose, arabinose, xylose, mannitol and dulcitol, but not adonitol, inositol or salicin, are fermented with acid and gas production. Acetyl-methyl-carbinol is not formed from glucose. Milk becomes acid, reverting to alkaline. Is not agglutinated by *Sal. enteritidis* serum. Found in the human intestines. A cause of enteric fever.

10. *Salmonella enteritidis* (Gärtner) Castellani and Chalmers, 1919.

Alternative: 33. *Bacterium enteritidis* (Gärtner) Chester, 1897.

SYNONYMY: *Bacillus enteritidis* Gärtner, 1888; *Klebsiella enteritidis* DeToni and Trevisan, 1889; *Bacillus Gaertner* Morgan, 1905.

Gärtner (1888) isolated this organism from the flesh and organs of a cow which had been killed because of an attack characterized by mucous diarrhoea, and from the spleen of a man who died after eating some of the flesh of the animal. Its description with regard to its morphology, staining reactions, and cultural characters was typical of the colon-typhoid bacteria. Gärtner named it *Bacillus enteritidis*.

While the organism has been considered a distinct species by all authors who have described it, means of differentiating it clearly from other members of the intermediate group have been developed comparatively recently. Its quick reversion of litmus milk from an acid to an alkaline reaction was used to separate it from *Sal. paratyphi* (paratyphoid A), (Schottmüller, 1901; Kayser, 1904; Morgan, 1905). Ford (1905), Harding and Ostenberg (1912), and Krumwiede, Pratt and Kohn (1916) found that *Sal. enteritidis* was one of a group which differed from paratyphoids in their ability to produce acid and gas from xylose.\*

The agglutination test served for a long time to distinguish *Sal. enteritidis* from *Sal. schottmülleri* (paratyphoid B) and from *Sal. cholerae suis*. Jordan (1917) separated *Sal. schottmülleri* and *Sal. enteritidis* from *Sal. cholerae suis* by their action on dulcitol and arabinose, the first two fermenting both sugars rapidly with acid and gas production, while *Sal. cholerae suis* fermented them slowly or not at all. In the same year, Weiss and Rice used inositol to differentiate *Sal. enteritidis* from *Sal. schottmülleri*. *Sal. enteritidis*, as well as *Sal. cholerae suis* and *Sal. abortivo-equinum*, were non-gas-producing on this sugar; *Sal. schottmülleri*, gas-producing. The species as it is generally recognized today is adequately defined by Winslow, Kligler and Rothberg (1919).

\* Jordan and Victorsan (1917) used blackening of lead-acetate agar for differentiation of *enteritidis* from *paratyphi*, the former giving a positive reaction, the latter, negative.

**Specific Diagnosis:** A motile rod, conforming to the generic diagnosis. Levulose, maltose, arabinose, xylose, mannitol and dulcitol are fermented with acid and gas production; adonitol, inositol and salicin are not attacked. Acetyl-methylcardinol is not formed from glucose. Litmus milk becomes acid, reverting rapidly to alkalinity. Found in human and animal intestines, and sometimes in meat. One of the causes of epidemic meat poisoning.

11. *Salmonella abortivo-equina* (Good and Corbett) Bergey et al, 1923.

Alternative: 34. *Bacterium abortivo-equinum* (Good and Corbett) Fitch, 1920.

**SYNONYMY:** *Bacillus abortivus equinus* Good and Corbett, 1913; *Bacillus abortus equi* Meyer and Boerner, 1913; *Bacillus abortivo-equinus* Good and Corbett, 1916; *Bacillus abortus equinus* Weiss and Rice, 1917; *Bacterium abortum-equi* Holland, 1920; *Salmonella abortus-equi* (Meyer and Boerner) Bergey et al, 1925.

The name *Bacillus abortivus equinus* was given to the organism causing infectious abortion of mares, by Good and Corbett in 1913; Meyer and Boerner in the same year proposed the name *Bacillus abortus equi*. Good and Corbett's name, however, antedates that of Meyer and Boerner by six months. They were all working with the same organism, an undoubted member of the intermediate or *Salmonella* group. The morphological and cultural characters were those of the organisms of the colon-typhoid series. Glucose, mannitol, dulcitol, xylose were among the sugars fermented with acid and gas; lactose and sucrose were not attacked. Good and Corbett (1916) and Good and Smith (1916) reported slight fermentation with gas production in both lactose and sucrose. It is significant, however, that they also found slight gas production in these sugars by *Sal. enteritidis*. This would seem to indicate a probable partial inversion of their disaccharids. No other author has reported gas production for *Sal. abortivo-equina* in these two sugars. Considerable cross-agglutination occurred with other members of the *Salmonella* group, especially with *Sal. enteritidis*. Weiss and Rice (1917) tested the organism in inositol and found it non-gas-producing.

Good and Corbett (1916) corrected the name from trinominal form by changing it to *Bacillus abortivo-equinus*.

**Specific Diagnosis:** A motile rod, conforming to the generic diagnosis. It produces acid and gas from levulose, maltose, arabinose, xylose, mannitol and dulcitol; dextrin, adonitol, inositol and salicin are not fermented. Litmus milk becomes acid, then alkaline. It may be differentiated from *Salmonella enteritidis* by means of the agglutinin-absorption test. The cause of infectious abortion of mares.

12. *Salmonella paratyphi* (Kayser) Bergey et al, 1923.

Alternative: 35. *Bacterium paratyphi* Kayser, 1902.

**SYNONYMY:** *Bacterium paratyphi* Type A, Kayser, 1902; "Bacillus paratyphoid A. Schottmüller" Morgan, 1905; *Bacillus paratyphosus* A, Wilson, 1908; *Bacillus paratyphosus* Winslow, Kligler, Rothberg, 1919; *Salmonella para-typhosus* A, (Schottmüller) Castellani and Chalmers, 1919.

The term "paratyphosus A" has been variously accredited to Brion and Kayser (1902) and to Schottmüller (1901). The organism now commonly known under this name was described by the above authors, but was



not named by them. Kayser (1902) used for paratyphoid organisms (both A and B) the name *Bacterium paratyphi*. In 1904, he separated *Bacterium paratyphi* into types A and B, (Type A for the Brion and Kayser organism and two of Schottmüller's organisms) on the basis of their action in litmus milk and on agglutination tests. Type A produced and maintained an acid reaction in milk, while Type B reverted in two weeks to an alkaline reaction. (Boycott (1906) and others have shown that para A milk cultures eventually become alkaline.) Neither type was able to attack lactose.

The organisms have been recognized as distinct and recognizable species since the time of their first isolation. Subsequent authors working with subcultures of the original strains and with freshly isolated strains have added much to their characterization. Ford (1905) noted the inability of para A to attack xylose and suggested this character for differentiation from para B. Harding and Ostenberg (1912) and Krumwiede, Pratt and Kohn (1916) also favored xylose fermentation for differentiation of the para B-*enteritidis-cholerae suis* types from para A types. Wiess and Rice (1917) pointed out that para A fails to attack inositol while para B produces acid and gas. The species is, at the present time, very clearly distinguished from related species of the intermediate group.

In order that the species might have a name in proper Latin form, Winslow, Kligler and Rothberg (1919) proposed *Bacillus paratyphosus*. It would seem, however, that Kayser's name, *paratyphi*, might be retained for the type A since it was applied to both species before they were differentiated. This would be in accordance with the Botanical Rules of Nomenclature. (Article 47. When a species or subdivision of a species is divided into two or more groups of the same nature, if one of the two forms was distinguished or described earlier than the other, the name is retained for that form.)

**Specific Diagnosis:** A motile rod, conforming to the generic diagnosis. Levulose, maltose, arabinose, mannitol and dulcitol are fermented with acid and gas production; raffinose, xylose, adonitol, inositol and salicin are not attacked. Acetyl-methyl-carbinol is not produced from glucose. Indol is not produced. Litmus milk becomes acid, reverting very slowly to alkalinity. The cause of paratyphoid fever in man.

13. *Salmonella woliniæ* (Castellani) Castellani and Chalmers, 1919.

Alternative: 36. *Bacterium woliniæ* (Castellani) Weldin and Levine, 1923.

**SYNONYMY:** *Bacillus woliniæ* Castellani, 1917.

Castellani (1917) described this organism as a motile rod, Gram-negative, not liquefying gelatin nor serum, not producing indol, producing acid and gas from glucose, maltose and mannitol, acid, or acid and gas from galactose, and an acid or alkaline reaction in saccharose. Levulose, lactose, dulcitol, dextrin, raffinose, arabinose, adonitol, inulin, sorbitol, inositol and salicin were not attacked. Litmus milk was made acid and sometimes reverted to alkalinity.

The organism is distinctive by reason of its inability to attack levulose. On this account it is deemed worthy of recognition as a species. Weldin and Levine (1923) placed it in their subgenus *Salmonella* of the genus *Bacterium*.



**Specific Diagnosis:** A motile rod, conforming to the generic diagnosis. It ferments maltose and mannitol with acid and gas, but does not attack levulose, raffinose, arabinose, dextrin, dulcitol, adonitol, inositol or salicin. Indol is not produced. Litmus milk becomes acid, sometimes reverting slowly to alkalinity. Found in the human intestinal tract.

14. *Salmonella watareka* (Castellani) Bergey et al, 1923.

Alternative: 37. *Bacterium watareka* (Castellani) Weldin and Levine, 1923.

SYNONYMY: *Bacillus watareka* Castellani, 1917.

Castellani (1917) described this organism as a motile rod, Gram-negative, not liquefying gelatine nor serum, producing acid in litmus milk, producing indol, producing acid and gas from a number of carbohydrates, including glucose, maltose, dulcitol, mannitol, raffinose, arabinose, sorbitol and inositol. Lactose, sucrose, dextrin, adonitol, inulin and salicin were not attacked.

The organism is evidently a member of the intermediate group. Its acid production without reversion to alkalinity in litmus milk allies it to the paratyphoid A organism. Its ability to attack inositol and raffinose, and to produce indol, however, distinctly separate it from this bacillus. Similar organisms have been described by MacConkey (1905), Cathcart (1906), Morgan (1907) and Lewis (1911).

Weldin and Levine (1923) included it in their subgenus *Salmonella* of the genus *Bacterium*.

**Specific Diagnosis:** A motile rod, conforming to the generic diagnosis. Acid and gas are produced from levulose, maltose, raffinose, arabinose, mannitol, dulcitol and inositol, but not from adonitol or salicin. Indol is produced. Litmus milk becomes acid. Found in the intestinal tract of man.

15. *Salmonella pullorum* (Rettger) Bergey et al, 1923.

Alternative: 38. *Bacterium pullorum* Rettger, 1909.

SYNONYMY: *Bacillus pullorum* Smith and TenBroeck, 1917; *Salmonella pullorum* (Rettger) Bergey et al, 1925.

Rettger first described this organism in 1900 and 1901. It was further described by Rettger and Harvey in 1908, but it was not named until 1909, when Rettger named it *Bacterium pullorum*. The bacillus was shown to be the cause of bacillary white diarrhoea of chicks. Its morphological and cultural characters were typical of the colon-typhoid series. At first reported motile, it was later (Rettger and Harvey, 1908) found to be non-motile. It fermented dextrose and mannitol with acid and gas production, but not maltose, lactose, saccharose, inulin nor dextrin.

There seems to have been considerable confusion for a time between this organism and the fowl-typhoid bacillus. Various authors, notably Smith and TenBroeck (1915), Goldberg (1917), Rettger and Koser (1917), and Hadley, Caldwell, Elkins and Lambert (1917), compared the two organisms. While they found the two closely related serologically, they all found behavior in carbohydrate media an easy method of differentiation. *Sal. pullorum* produced gas as well as acid from certain carbohydrates, while the fowl typhoid organism produced acid only. Further, *pullorum*

was found to be strictly maltose-dextrin-dulcitol negative, while the other organism attacked these substances.

Hadley, Elkins and Caldwell (1918) described *Sal. pullorum* as "weakly xylose-positive" (acid and gas). Krumwiede, Kohn and Valentine (1918) recorded acid production from xylose as  $+$  or  $\pm$ . Mulsow (1919) reported gas variable and acid positive for xylose. Apparently the organism should be considered as usually attacking xylose.

**Specific Diagnosis:** A non-motile rod, conforming to the generic diagnosis. Levulose, arabinose, mannitol and usually xylose are fermented with acid and gas production; maltose, raffinose, dextrin, dulcitol, adonitol, inositol and salicin are not attacked. Acetyl-methyl-carbinol is not produced from glucose. Indol is not produced. Litmus milk is acidified, slowly reverting to neutral or alkaline reaction. The cause of "white diarrhoea" in chicks.

16. *Salmonelli morganii* (Winslow, Kligler, Rothberg) Castellani and Chalmers, 1919.

Alternative: 39. *Bacterium morganii* (Winslow, Kligler and Rothberg) Holland, 1920.

SYNONYMY: Organism No. 1, Morgan, 1906; *Bacillus morgan* No. 1, Castellani and Chalmers, 1919; *Bacillus morgani* Winslow, Kligler, Rothberg, 1919; *Bacillus pseudo-morgani* Castellani and Chalmers, 1919.

Morgan (1906) first described this organism which he had isolated from the stools from cases of summer diarrhoea in infants. He described it (Organism No. 1) as a motile bacillus closely resembling the hog cholera bacillus of McFadyean, but differing from the latter in its alkaline reaction on litmus milk, its greater indol production and its failure to attack maltose, arabinose and dextrin. The monosaccharids were the only carbohydrates which it was able to attack. Morgan felt on account of its prevalence that it probably had some etiological significance in infant diarrhoea.

The organism has been repeatedly isolated from cases of diarrhoea in children (Lewis, 1911; Cox, Lewis and Glynn, 1912; Pirie, 1917; Stewart, 1917; Zironi and Capone, 1917; Tribondeau and Fichet, 1916; Logan, 1916; Thjotta, 1920; Levine, Ajwani and Weldin, 1925, etc.) both motile and non-motile varieties having been found. Winslow, Kligler and Rothberg (1919) concluded that "it constitutes a fairly definite type of common occurrence in the human intestinal canal" and gave it the name *Bacillus morgani*.

**Specific Diagnosis:** A rod conforming to the generic diagnosis. Both motile and non-motile varieties. Acid and gas produced from monosaccharids, and rarely from xylose; maltose, raffinose, arabinose, dextrin, mannitol, dulcitol, inositol, salicin, adonitol and sorbitol are not attacked. Litmus milk remains neutral or becomes alkaline. Indol is produced. Found in normal and diarrheal stools, particularly of infants.

17. *Salmonella foetida* (Perez) Bergey et al, 1923.

Alternative: 40. *Bacterium foetidum* (Perez) Weldin and Levine, 1923.

SYNONYMY: *Coccobacillus foetidus ozenae* Perez, 1899; *Coccobacillus (foetidus) ozaenae* Ward, 1917; *Escherichia foetida* Bergey et al, 1925.

Perez (1899) described this organism, isolated by him from ozena, under the name *Coccobacillus foetidus ozenae*. The bacillus was small, often coccoid in form, though it might show filaments. It was non-spore-forming, non-motile, easily cultivated and easily stained, though not by Gram's method. On ordinary media its growth was typical of the colon-typhoid series. Neither gelatin nor blood serum were liquefied. Indol was formed. Milk was never coagulated, nor was lactose fermented. It produced a pronounced and characteristic foetid odor. It was pathogenic for guinea pigs, mice, pigeons and rabbits.

Ward (1917) studied a number of strains of Perez' bacillus, both from European sources and from his own isolations. Besides confirming Perez' findings, he investigated its action on a number of carbohydrates: glucose, levulose, maltose, dextrin, lactose, saccharose, glycerol, inulin and mannitol. Of these, glucose was the only one attacked; gas production even in this sugar was slow, but might after a number of days amount to 15-75% ( $\text{CO}_2$  and  $\text{H}_2$ ).

Weldin and Levine (1923) listed the organism in the genus *Bacterium*, subgenus *Salmonella*. Bergey *et al* (1923) included it in their genus *Salmonella*, but in the second edition of their Manual (1925) moved it to the genus *Escherichia*. Just why it was placed in this group is not apparent, since its fermentation reactions as determined by Ward (1917) definitely place it with *Salmonella*.

**Specific Diagnosis:** A non-motile rod, conforming to the generic diagnosis. As indicated above, of the carbohydrates tested, glucose is the only one which is fermented with acid and gas. Indol is produced. Litmus milk becomes slightly acid. Found in the nasal exudate from cases of ozena.

18. *Salmonella giumai* (Castellani) Bergey *et al*, 1923.

Alternative: 41. *Bacterium giumai* (Castellani) Weldin and Levine, 1923.

SYNONYMY: *Bacillus giumai* Castellani, 1912; *Wesenbergus giumai* (Cast.) Castellani and Chalmers, 1919.

This organism was described by Castellani (1912) as a rather rare cause of parenteric fever in the tropics. While its cultural and morphological characters were not given in detail, they evidently were the same as those of the colon-typhoid series with which it was grouped. It was non-motile, did not liquefy gelatin or serum, produced indol, brought about an acid reaction followed by alkalinity in litmus milk and fermented a number of carbohydrates with acid and gas, including glucose, maltose, dextrin, arabinose, sorbitol, galactose, levulose, salicin and glycerol. Acid but not gas was produced from lactose. Saccharose, dulcitol, mannitol, raffinose, adonitol, inulin and inositol were not fermented.

Evidently the organism belongs in the *Salmonella* group. Its failure to attack mannitol places it in the Morgan section, but its greater fermentative ability adequately separates it from the Morgan bacillus.

**Specific Diagnosis:** A non-motile rod, conforming to the generic diagnosis. It produces acid and gas from levulose, maltose, arabinose, dextrin, salicin and sorbitol; acid, but not gas, is produced from lactose (?); raffinose, mannitol, dulcitol, adonitol and inositol are not attacked. Litmus milk becomes acid followed by alkalinity. Indol is produced. Found in the intestines of man. A cause of parenteric fever.

19. *Salmonella macfadyeanii* (Weldin and Levine) Comb-nov.

Alternative: 42. *Bacterium macfadyeanii* Weldin and Levine, 1923.

The writer, up to the present time, has been unable to secure McFadyean's original description of this organism. It was described by Morgan in 1905 and by Castellani in 1912, under the title "B-hog cholera, McFadyean." Morgan (1906, 1907) in discussing his organism No. 1, (*Sal. morganii*), stated that it resembled the hog cholera bacillus of McFadyean more than any other known pathogen. The organism is of interest as being, perhaps, the first described of the non-mannitol-fermenting members of the Intermediate section. It is somewhat more active than the Morgan bacillus.

**Specific Diagnosis:** A motile rod, conforming to the generic diagnosis. It ferments levulose, maltose, arabinose and dextrin with acid and gas production; raffinose, mannitol, dulcitol, salicin and sorbitol are not attacked. Indol is produced. Litmus milk becomes acid. Found in the intestines of hogs.

20. *Salmonella archibaldii* (Castellani) Castellani and Chalmers, 1919.

Alternative: 43. *Bacterium archibaldii* (Castellani) Weldin and Levine, 1923.

SYNONYMY: *Bacillus archibaldi* Castellani, 1917; *Aerobacter archibaldi* (Castellani and Chalmers) Bergey *et al.*, 1923.

This organism was isolated from cases of parenteric fever and described by Archibald (1912). Castellani (1917) gave it the name of *Bacillus archibald* (later changed to *archibaldi*). It evidently resembled in its morphological and cultural characters, the organisms of the colontyphoid series. Castellani described it as a Gram-negative bacillus not liquefying gelatin nor serum. In litmus milk, it produced acidity followed by alkalinity. Glucose, maltose, dulcitol, mannitol and sorbitol were fermented with acid and gas, but lactose, saccharose, raffinose and adonitol were not attacked. It produced indol and gave a positive Voges-Proskauer reaction. This last named property sets it apart from the other members of the *Salmonella* group. Buchan (1910) has likewise described organisms of the intermediate group giving the Voges-Proskauer reaction. Although this type of organism is unusual, they evidently do exist and should, therefore, be recognized.

**Specific Diagnosis:** A motile rod, conforming to the generic diagnosis. Maltose, mannitol, dulcitol and sorbitol are fermented with acid and gas; raffinose and adonitol are not attacked. Acetyl-methyl-carbinol is produced from glucose. Indol is produced. Litmus milk becomes acid, followed by alkalinity. Isolated from human intestines. A probable cause of enteric fever.

GENUS 5. *EBERTHELLA* Buchanan, 1918.

SYNONYMY: *Dysenteroides* Castellani and Chalmers, 1919; *Eberthus* Castellani and Chalmers, 1919; *Lankoides* (in part) Castellani and Chalmers, 1919.

This name was first used by Buchanan (1918) for the third subgenus of his genus *Bacterium* with the following definition:

Not producing gas from any of the carbohydrates, acid may or may not be formed. Type species: *Bacterium (Eberthella) typhi* Flüge.



Weldin and Levine (1923) likewise used it for a subgenus of *Bacterium*, its distinguishing character being the production of acid but not gas from glucose.

Bergey *et al* (1923) elevated the subgenus to generic rank. They defined it (1925) as follows:

Motile or non-motile rods, generally occurring in the intestinal canal of man, usually in different forms of enteric inflammation. Attack a number of carbohydrates with the formation of acid but no gas. Do not form acetyl-methyl-carbinol. Type species: *Eberthella typhi* (Eberth-Gaffky) Buchanan.

The organisms thus characterized form a clearly defined group of the colon-typhoid series and should be recognized as such. In reality, two rather distinct types are represented: namely, typhoid and dysentery. The typhoid and dysentery bacilli produce characteristically different diseases, show little or no serological relation, the former being motile, the latter, non-motile. It is proposed to use the term *Eberthella* for the fifth genus of the colon-typhoid series, to include only the motile forms of the typhoid-dysentery section. The non-motile forms will be included in the genus *Shigella*.

**Generic Diagnosis:** Motile, Gram-negative, non-spore-forming rods. Produce acid but not gas from glucose. Generally occur in the intestinal tract of man or animals, usually in enteric inflammation.

Type species: *Eberthella typhosa* (Zopf).

#### KEY TO SPECIES OF THE GENUS *EBERTHELLA*.

- a. Neither acid nor gas produced from lactose.
  - b. Neither acid nor gas produced from inositol.
    - c. Acid, but not gas, produced from levulose, maltose and dextrin.
      - d. Acid, but not gas, produced from mannitol and sorbitol; neither acid nor gas from inulin and salicin.
        - 1. *Eberthella typhosa*.
    - dd. Neither acid nor gas produced from mannitol and sorbitol; acid, but not gas, from inulin and salicin.
      - 2. *Eberthella pritzitzi*.
  - cc. Neither acid nor gas produced from levulose, maltose and dextrin.
    - 3. *Eberthella lewisii*.
- bb. Acid, but not gas, produced from inositol.
  - c. Neither acid nor gas produced from salicin; indol not produced.
    - 4. *Eberthella kandiensis*.
  - cc. Acid, but not gas, produced from salicin; indol produced.
    - 5. *Eberthella talavensis*.
- aa. Acid, but not gas, produced from lactose.
  - b. Acid, but not gas, produced from mannitol and arabinose.
    - c. Acid, but not gas, produced from dulcitol.
      - 6. *Eberthella pyogenes*.



cc. Neither acid nor gas produced from duleitol.

d. Neither acid nor gas produced from salicin; indol produced.

7. *Eberthella belfastiensis*.

dd. Acid, but not gas, produced from salicin; indol not produced.

8. *Eberthella wilsonii*.

bb. Neither acid nor gas produced from mannitol and arabinose.

9. *Eberthella bentotensis*.

#### SPECIES OF *EBERTHELLA*

1. *Eberthella typhosa* (Zopf) Comb. nov.

Alternative: 43. *Bacterium typhosum* Zopf, 1884.

SYNONYMY: *Bacillus typhosus* Eberth, Zopf, 1885; *Bacillus typhi abdominalis* Flügge, 1886; *Bacillus typhi* (Eberth, Gaffky) Schroeter, 1889; *Vibrio typhosus* Trevisan, 1889; *Bacterium typhi*, Eberth, Gaffky-Lehmann and Neumann, 1896; *Eberthus typhosus* (Eberth) Castellani and Chalmers, 1919; *Eberthella typhi* (Eberth-Gaffky) Castellani and Chalmers, Bergey *et al*, 1923; *Eberthella typhi* (Schroeter) Buchanan, Bergey *et al*, 1925; not *Micrococcus ileotyphi* Trevisan, 1879; *Bacillus typhosus* Klebs, 1881; *Mikrokokkus typhi abdominalis* Letzerich 1881; *Metallacter ileotyphi* Trevisan, 1882.

Eberth (1880) is commonly credited as being the first to demonstrate the presence in the body of typhoid cadavers of the organism now known to be the cause of the disease. The organism was first isolated by Gaffky in 1884. Neither author gave to the bacillus any specific name. Eberth (1880) recorded finding in some cells "sporenähnlicher Körperchen" and Gaffky (1884) definitely described the organism as forming spores which were particularly abundant on potato cultures grown at 30 to 42° C. Otherwise their descriptions, though brief, were typical of the typhoid bacillus. Subsequent investigators believed Eberth and Gaffky to have been mistaken as to the presence of spores. Sternberg (1892) said, "Spherical or oval refractive granules are often seen at the extremities of the rods, especially in potato cultures kept in the incubating oven; these are not reproductive spores, as was at first supposed."

Klebs in 1881 discovered an organism which he thought was the causative agent of typhoid fever and which he called *Bacillus typhosus*. It was described as threads sometimes 50 microns long and was unquestionably a spore former. Trevisan (1879) used the name *Micrococcus ileotyphi* for an organism supposedly the cause of abdominal typhoid. His description was too brief for any possible recognition of the organism. In 1882, he transferred the species to the genus *Metallacter*. Judging from his discussion, he considered his organism identical with Klebs' *Bacillus typhosus*. In his I Generi e le Specie delle Batteriacee, 1889, we find the following:

"*Bacillus Klebsii* Trev., 1885 (*Bacillus typhosus* Klebs, 1881)—Bacillo della necrosi intestinale"—, and further:

"*Vibrio typhosus* Trev. (*Bacillus typhosus* Eberth, 1880). Anaerobio facultativo. Non liquefa la gelatina."

Letzerich (1881) used the name *Mikrokokkus typhi abdominalis* for an organism which, as described in the body of his article, might have been the typhoid bacillus. In a "Nachschrift," however, he recorded finding long spore forming filaments and concluded his organism to be identical with Klebs' *Bacillus typhosus* which had been described some months previously.

Zopf (1884) used the name *Bacterium typhosum* for Eberth's organism, with a very brief description. This was apparently the first valid name given to the true typhoid organism. In the third edition of "Die Spaltpilze," published in 1885, he changed the name to *Bacillus typhosus*.

Flügge (1886) used the term *Bacillus typhi abdominalis*. He referred to both Eberth and Klebs organisms.

*Bacillus typhi* was used by Schroeter (1889) for Eberth and Gaffky's organism. The specific name *typhi* has been used extensively for the organism, with credit given variously to Eberth, Gaffky and to Schroeter.

The organism has been carefully studied by subsequent investigators and its cultural, biochemical and serological characters fully determined. Winslow, Kligler and Rothberg (1919) gave a compact and accurate summary of its characters under the name *Bacillus typhosus* (Zopf).

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**Specific Diagnosis:** A rod, conforming to the generic diagnosis, single or in pairs, occasionally in short chains. It produces acid, but not gas, from levulose, maltose, dextrin, mannitol and sorbitol; lactose, sucrose, inulin, inositol, salicin and usually arabinose and dulcitol, are not fermented. Litmus milk is made acid, slowly reverting to neutral or slight alkalinity. Indol is not produced. Pathogenic for man and laboratory animals. The cause of typhoid fever in man.

## 2. *Eberthella pritznitzi* (Castellani) Comb. nov.

Alternative: 44. *Bacterium pritznitzi* (Castellani) Weldin and Levine, 1923.

SYNONYMY: *Bacillus pritznitzi* Castellani, 1917; *Eberthus pritznitzi* (Cast.) Castellani and Chalmers, 1919; *Eberthus pritznitzi* (Cast.) Castellani and Chalmers, 1920; *Bacterium pritznitzi* Weldin and Levine, 1923.

Castellani (1917) described this organism as a motile rod, Gram-negative, not liquefying gelatin or serum, indol production negative, and producing acid in litmus milk. It produced acid, but not gas, from glucose, maltose, inulin, salicin and dextrin. Lactose, sucrose, dulcitol, mannitol, raffinose, arabinose, adonitol, sorbitol and inositol were not attacked. It was the causal organism of some cases of parenteric fever.

This organism resembles *Eberthella typhosa* very closely, differing in its action on mannitol, sorbitol, inulin and salicin. Weldin and Levine (1923) included it in their subgenus *Eberthella* of the genus *Bacterium*.

**Specific Diagnosis:** A rod conforming to the generic diagnosis. Levulose, maltose, dextrin, inulin and salicin are fermented with acid production; it does not attack lactose, sucrose, raffinose, arabinose, mannitol, dulcitol, sorbitol or inositol. Litmus milk is acidified. Indol is not produced. A cause of paraenteric fever.

3. *Eberthella lewisii* (Weldin and Levine) Comb. nov.

Alternative: 45. *Bacterium lewisii* Weldin and Levine, 1923.

SYNONYMY: Organism B3<sub>2</sub>, Lewis, 1911.

Lewis (1911) described an organism isolated from the faeces of a normal child, as a non-chromogenic, Gram-negative, motile bacillus, not liquefying gelatin, not producing indol and making litmus milk alkaline. Of a list of 17 carbohydrates studied, only one was attacked, namely glucose, with acid production.

The fact that an organism exists of such weak fermentative ability that it is able to attack only a single sugar, would seem to be sufficient reason for its recognition as a species. Weldin and Levine (1923) created for this organism, which Lewis designated as B3<sub>2</sub>, the name *Bacterium lewisii*. They included it in their subgenus *Eberthella*.

**Specific Diagnosis:** A rod conforming to the generic diagnosis. Of the carbohydrates glucose, levulose, galactose, mannose, maltose, lactose, sucrose, raffinose, dextrin, inulin, mannitol, dulcitol, sorbitol, inositol and salicin, only glucose is fermented with acid production. Litmus milk is made alkaline. Indol is not produced. Found in human faeces.

4. *Eberthella kandiensis* (Castellani) Bergey *et al*, 1923.

Alternative: 46. *Bacterium kandiensis* (Castellani) Weldin and Levine, 1923.

SYNONYMY: *Bacillus kandiensis* Castellani, 1912; *Eberthus kandiensis* Castellani and Chalmers, 1919.

Castellani (1912) described this organism as a motile rod, Gram-negative, not liquefying gelatin nor serum, not producing indol, and giving an acid reaction in litmus milk followed by alkalinity. Acid, but not gas, was produced from glucose and some other carbohydrates. The organism was considered to be ordinarily non-pathogenic, but, under special conditions, it might become pathogenic, causing a type of parenteric fever.

The bacillus as described resembled the typhoid organism, differing from it by its action on some of the carbohydrates, particularly inositol, sucrose, maltose and dextrin. Weldin and Levine (1923) included it in their subgenus *Eberthella* of the genus *Bacterium*.

**Specific Diagnosis:** A rod conforming to the generic diagnosis. Acid, but not gas, is produced from levulose, sucrose (slight amount), mannitol and inositol; lactose, maltose, raffinose, arabinose, dextrin, inulin, dulcitol, sorbitol and salicin are not attacked. Litmus milk becomes acid, reverting to alkalinity. Indol is not produced. Sometimes pathogenic, causing a type of parenteric fever. Found in the human intestines.

5. *Eberthella talavensis* (Castellani) Bergey *et al*, 1923.

Alternative: 47. *Bacterium talavensis* (Castellani) Weldin and Levine, 1923.

SYNONYMY: *Bacillus talavensis* Castellani, 1912; *Eberthus talavensis* (Cast.) Castellani and Chalmers, 1919.

This organism as described by Castellani (1912) differed from *Eberthella kandiensis* (Cast.) (which see) by its production of indol, production of acid from salicin, and inability to attack mannitol, adonitol, isodulcitol

and erythritol. It was listed as one of a number of species which are the causal agents of enteroides.

Weldin and Levine (1923) placed it in their subgenus *Eberthella* of the genus *Bacterium*.

**Specific Diagnosis:** A rod conforming to the generic diagnosis. It produces acid, but not gas, from levulose, sucrose, inositol and salicin; lactose, maltose, raffinose, arabinose, dextrin, inulin, mannitol, dulcitol and sorbitol are not fermented. Litmus milk is made alkaline. Indol is produced. Found in the intestines of man. A possible cause of enteric fever.

6. *Eberthella pyogenes* (Passet) Bergey et al, 1923.

Alternative: 48. *Bacterium pyogenes* (Passet) Migula, 1900.

SYNONYMY: *Bacillus pyogenes foetidus* Passet, 1885; *Lankoides pyogenus foetidus* (Passet) Castellani and Chalmers, 1919; *Bacterium pyogenes-foetidum* (Passet) Holland, 1920; *Eberthella pyogenes* (Migula) Bergey et al, 1925.

Passet (1885) isolated this organism from a rectal abscess. He named it *Bacillus pyogenes foetidus* and described it as a small, motile rod with rounded ends. Its growth on ordinary laboratory media was typical of the colon-typhoid organisms. MacConkey (1905) obtained a culture of the organism from Krål and found it able to produce acid from glucose, levulose, galactose, maltose, arabinose, raffinose, lactose, sucrose, mannitol, sorbitol, dulcitol and dextrin. Castellani (1912) added to its description the ability to produce indol.

The organism is evidently a member of the genus *Eberthella*, characterized by its ability to produce acid in an exceptionally large number (for this group) of carbohydrates. Migula (1900) reduced the name to binominal form, calling it *Bacterium pyogenes*.

**Specific Diagnosis:** A motile rod conforming to the generic diagnosis. Acid, but not gas, is produced from levulose, lactose, maltose, sucrose, raffinose, arabinose, dextrin, mannitol, dulcitol and sorbitol. Indol is produced. Litmus milk becomes acid and coagulated. Isolated from a rectal abscess.

7. *Eberthella belfastiensis* (Wilson) Bergey et al, 1923.

Alternative: 49. *Bacterium belfastiensis* (Wilson) Weldin and Levine, 1923.

SYNONYMY: *Bacillus belfastiensis* II, Wilson, 1908.

Wilson (1908) described under the name *Bacillus belfastiensis* II, an organism isolated by Mair (1906) from the urine of a case of cystitis. Mair had not completely described nor had he named the bacillus. The organism was a motile non-sporing bacillus, Gram-negative, producing a greyish-white growth on agar and uniform turbidity in broth. Gelatin was not liquefied. No gas was produced in glucose. Indol was formed. Acid was produced in glucose, levulose, maltose, lactose, sucrose, mannitol, glycerin, arabinose, raffinose and sorbitol. Dulcitol, glycerol, adonitol, erythritol, salicin, dextrin and inulin were not attacked. Acid was produced in litmus milk.

The species is clearly differentiated from the typhoid bacillus by its acid production in lactose and sucrose. Weldin and Levine (1923) included it in their subgenus *Eberthella* of the genus *Bacterium*.



**Specific Diagnosis:** A rod conforming to the generic diagnosis. Levulose, lactose, maltose, sucrose, raffinose, arabinose, mannitol and sorbitol are fermented with acid, but not gas production; dextrin, inulin, dulcitol and salicin are not attacked. Indol is formed. Acid is produced in milk. Isolated from urine.

8. *Eberthella wilsonii* sp. nov.

Alternative: 50. *Bacterium wilsonii* sp. nov.

SYNONYMY: *Bacillus belfastiensis* V Wilson, 1908.

*Belfastiensis* V, isolated by Wilson (1908) from urine, was similar to his *Belfastiensis* II (*Eberthella belfastiensis*) differing from it by fermenting salicin and dextrin, but not attacking sorbitol or glycerin, and by its failure to produce indol. Since the name *belfastiensis* was used for the preceding organism, it is invalid here; the specific name *wilsonii* is suggested for this species.

**Specific Diagnosis:** A rod conforming to the generic diagnosis. Acid, but not gas, is produced from levulose, lactose, maltose, sucrose, raffinose, arabinose, dextrin, mannitol and salicin; neither acid nor gas are produced from inulin and dulcitol. Acid is produced from litmus milk. Indol is not formed. Found in urine.

9. *Eberthella bentotensis* (Castellani) Bergey et al, 1923.

Alternative: 51. *Bacterium bentotensis* (Castellani) Weldin and Levine, 1923.

SYNONYMY: *Bacillus bentotensis* Castellani, 1912; *Dysenteroides bentotensis* (Cast.) Castellani and Chalmers, 1919.

This organism was described by Castellani (1912) as a motile, Gram-negative rod, producing indol, not liquefying gelatin nor serum, and giving an acid reaction in litmus milk. Acid, but not gas, was produced from glucose, maltose, lactose, sucrose, dulcitol, raffinose, inositol, salicin and glycerin. Mannitol, dextrin, arabinose, adonitol, inulin and sorbitol were not attacked.

It differs from *Eberth. typhosa* in its ability to produce acid from lactose and from *Eberth. belfastiensis* and *Eberth. pyogenes* in not attacking mannitol or arabinose. It was included by Weldin and Levine (1923) in their subgenus *Eberthella* of the genus *Bacterium*.

**Specific Diagnosis:** A rod conforming to the generic diagnosis. Acid is produced from levulose, lactose, maltose, sucrose, raffinose, dulcitol, inositol and salicin; arabinose, dextrin, inulin, mannitol and sorbitol are not fermented. Acid is produced in litmus milk. Indol is formed. Found in human intestinal tract.

Genus 6. *SHIGELLA* Castellani and Chalmers, 1919.

SYNONYMY: *Graciloides* Castellani and Chalmers, 1919; *Lankoides* (in part) Castellani and Chalmers, 1919; *Eberthella* (in part) Buchanan, 1918.

Castellani and Chalmers (1919) used this term for the third genus of the tribe *Ebertheae*. The description of the genus was as follows:

*Ebertheae* non-motile, partially fermenting glucose with the production of acid but no gas; lactose not fermented. Milk not clotted. Type species: *Shigella dysenteriae* (Kruse, 1899).



Lactose fermentation and clotting of milk are not considered advisable characters to use for generic differentiation for the typhoid-dysentery group. (See *Dysenteroides* and *Lankoides*, pp. 127 and 124.)

In the discussion of the name *Eberthella*, the creation of two genera to represent the typhoid and dysentery organisms was advocated. The first was to include the motile species, producing typhoid-like infections, and was to be called *Eberthella*. The name *Shigella* appears to be the most suitable name for the non-motile types, most of which are associated with some form of bacillary dysentery.

The term *Shigella* was also used by Castellani and Chalmers (1919) for a subgenus of their genus *Shigella*. The subgenus included only the non-mannitol-fermenters, the mannitol fermenters being designated by the name *Flexnerella*. It is not felt that the name should be retained for this grouping. The mannitol fermenters and non-fermenters may be considered sections of the genus *Shigella*, under the terms *Flexner* section and *Shiga* section.

**Generic Diagnosis:** *Non-motile, Gram-negative, non-spore-forming, short rods. Produce acid, but not gas, from glucose. Generally occur in the intestinal tract of man or animals. Usually pathogenic, producing dysenteries or dysentery-like diseases.*

Type species: *Shigella dysenteriae* (Shiga) Castellani and Chalmers, 1919.

#### KEY TO SPECIES OF THE GENUS *SHIGELLA*

- a. Neither acid nor gas produced from mannitol.
  - b. Neither acid nor gas produced from sucrose, arabinose and dextrin.
    - c. Neither acid nor gas produced from rhamnose; indol not produced.
      - 1. *Shigella dysenteriae*.
    - cc. Acid, but not gas, produced from rhamnose; indol produced.
      - 2. *Shigella ambigua*.
    - bb. Acid, but not gas, produced from sucrose, arabinose and dextrin.
      - 3. *Shigella lunavensis*.
  - aa. Acid, but not gas, produced from mannitol.
    - b. Neither acid nor gas produced from lactose.
      - c. Neither acid nor gas produced from xylose.
        - 4. *Shigella paradysenteriae*.
      - cc. Acid, but not gas, produced from xylose.
        - d. Acid, but not gas, produced from dulcitol; salicin not fermented.
          - e. Acid, but not gas, produced from glycerol; indol produced.
            - 5. *Shigella alkalescens*.
          - cc. Neither acid nor gas produced from glycerol; indol not produced.
            - 6. *Shigella gallinarum*.

- dd. Neither acid nor gas produced from dulcitol; acid produced from salicin.
- e. Acid, but not gas, produced from arabinose and maltose.
- 7. *Shigella pfaffii*.
- ee. Neither acid nor gas produced from arabinose and maltose.
- 8. *Shigella rettgeri*.
- bb. Acid, but not gas, produced from lactose.
- c. Acid, but not gas, produced from arabinose and rhamnose.
- d. Acid, but not gas, produced from xylose; indol usually produced.
- e. Neither acid nor gas produced from dulcitol.
- 9. *Shigella madampensis*.
- ee. Acid, but not gas, produced from dulcitol.
- 10. *Shigella ceylonensis*.
- dd. Neither acid nor gas produced from xylose; indol not produced.
- 11. *Shigella sonnei*.
- cc. Neither acid nor gas produced from arabinose and rhamnose.
- 12. *Shigella equi*.

#### SPECIES OF *SHIGELLA*

1. *Shigella dysenteriae* (Shiga) Castellani and Chalmers, 1919.

Alternative: 52. *Bacterium dysenteriae* (Shiga) LeBlaye and Gugenheim, 1914.

SYNONYMY: *Bacillus* of Japanese dysentery, Shiga, 1898; *Bacillus dysenteriae* Shiga, 1898; *Bacillus Shigae* Chester, 1901; *Bacillus dysentericus* (Shiga-Kruse) Ruffer and Willmore, 1909; *Eberthella dysenteriae* Cast. and Chalm., Bergey *et al*, 1923; *Eberthella dysenteriae* (Shiga) Bergey *et al*, 1925; not *Bacillus dysenteriae liquefaciens* Kruse, 1896; *Bacterium dysenteriae liquefaciens* (Ogata) Chester, 1897; *Bacillus dysenteriae* (Kruse) Migula, 1900.

Shiga (1898) first isolated this organism from cases of dysentery in Japan. He described it as a Gram-negative, non-spore-forming rod, very similar to the typhoid bacillus. He considered it as being motile, but Kruse (1900) and all later observers agree that it is non-motile. Its growth on agar, gelatin and potato was like that of the typhoid organism. Gelatin was not liquefied, milk was not coagulated, indol was not produced and no gas was formed from glucose. Subsequent investigators have shown it to produce acid from glucose, levulose and galactose, but not from lactose, sucrose, mannitol, dulcitol, xylose, raffinose, rhamnose, arabinose, maltose, salicin, inulin, inositol nor sorbitol.

Shiga (1898) named the organism *Bacillus dysenteriae*. Ogata (1892) had isolated an organism, which was listed by Flügge (1896) under the name *Bacillus dysenteriae liquefaciens*. It was a Gram-positive, gelatin liquefying organism, evidently not a member of the colon-typhoid series.

The name, however, was shortened by Migula (1900) to *Bacillus dysenteriae*. Chester (1901) followed Migula's use of the name *dysenteriae* for the organism of Ogata and called Shiga's organism *Bacillus Shigae*, and this name has been used extensively. The proper specific designation for the Shiga organism would seem to be that given by Shiga himself.

**Specific Diagnosis:** A short rod, 0.4 to 0.6 $\mu$  broad and 1.0 to 1.5 $\mu$  long, conforming to the generic diagnosis. It does not produce acid nor gas from lactose, maltose, sucrose, arabinose, xylose, dextrin, mannitol, dulcitol, salicin or rhamnose. Indol is not produced. Litmus milk becomes slightly acid, then neutral or alkaline. Found in the stools and intestines of humans. The cause of one form of dysentery.

## 2. *Shigella ambigua* (Andrews) Comb. nov.

Alternative: 53. *Bacterium schmitzii* Weldin and Levine, 1923.

**SYNONYMY:** Schmitz bacillus, Schmitz, 1917; *Bacillus ambiguus* Andrews, 1918; *Bacillus dysenteriae* "Schmitz", Murray, 1918; *Bacterium ambiguum* Levine, 1920; *Eberthella ambigua* (Andrews) Bergey *et al*, 1923; not *Bacterium ambiguum* Chester, 1899.

Schmitz (1917) isolated from a number of cases of dysentery, an organism which he called the Schmitz Bacillus. It was similar to the Shiga Bacillus, but differed from it in being able to produce indol. Mannitol, lactose, sucrose and maltose were not attacked. Andrews (1918) described an organism under the name *Bacillus ambiguus*, which did not differ in any significant way from the Schmitz bacillus. Murray (1918) came to the conclusion that Andrews' and Schmitz' organisms were identical. Levine (1920) reporting on five strains (designated as *B. ambiguus* and secured from Andrews) found none able to ferment mannitol, lactose, glycerol, dulcitol, sucrose, xylose or dextrin. Acid was produced from rhamnose by all, and indol was produced. These last two properties served to differentiate the organism from *Shigella dysenteriae*.

Andrews' specific name "*ambiguus*" is valid when used with the generic name "*Shigella*". *Bacterium ambiguum*, however, cannot be used as an alternative name for this species, since this name was applied by Chester (1899) to a motile, gelatin-liquefying, non-acid-producing organism. Weldin and Levine (1923) used the term *Bacterium schmitzii*.

**Specific Diagnosis:** A short rod, conforming to the generic diagnosis. It does not attack lactose, maltose, sucrose, xylose, dextrin, glycerol, mannitol or dulcitol. Acid is produced from rhamnose. Litmus milk becomes acid, followed by a neutral or alkaline reaction. Inagglutinable by Shiga immune serum. Found in human stools. The cause of one type of dysentery of man.

## 3. *Shigella lunavensis* (Castellani) Castellani and Chalmers, 1919.

Alternative: 54. *Bacterium lunavensis* (Castellani) Weldin and Levine, 1923.

**SYNONYMY:** *Bacillus lunavensis* Castellani, 1912.

This organism was described by Castellani (1912) as a non-motile rod, Gram-negative, producing indol and not liquefying gelatin nor serum. Acid was produced from glucose, sucrose, maltose, dextrin and arabinose. Lactose, dulcitol, mannitol, raffinose, adonitol, inulin, sorbitol, inositol and salicin were not attacked. The organism is similar to *Shigella dysenteriae*

in not fermenting mannitol, but differs in its production of indol and fermentation of sucrose, arabinose and dextrin.

Weldin and Levine (1923) included it in their subgenus *Eberthella* of the genus *Bacterium*. Similar organisms have been described by Mackie (1919) and DeBains (1917).

**Specific Diagnosis:** A short rod, conforming to the generic diagnosis. Acid is produced from maltose, sucrose, arabinose and dextrin, but not from lactose, mannitol, dulcitol or salicin. Indol is produced. Litmus milk becomes slightly acid, reverting to a slight alkaline reaction. Found in human stools.

#### 4. *Shigella paradysenteriae* (Collins) Comb. nov.

Alternative: 55. *Bacterium paradysenteriae* (Collins) Comb. nov.

**SYNONYMY:** *Bacillus dysenteriae* Flexner type, Strong type, Y type; *Bacillus paradysenteriae* Collins, 1905; *Shigella dysenteriae* (Flexner) (Hiss and Russell) (Strong) Castellani and Chalmers, 1919; *Bacillus flexneri* Levine, 1920; *Bacterium flexneri* Levine, 1920; *Bacterium dysenteriae* (Flexner) Holland, 1920; *Bacterium* (*Eberthella*) *flexneri* Weldin and Levine, 1923; *Eberthella paradysenteriae* (Flexner) (Hiss) (Strong) Bergey *et al*, 1923; *Eberthella paradysenteriae* (Collins) Bergey *et al*, 1925.

In March, 1900, Flexner isolated from cases of dysentery an organism which he believed to be the cause of the disease and which has since been commonly designated as *Bacillus dysenteriae* Flexner. Shiga had previously (1898) isolated a dysentery organism. At first both Flexner and Shiga believed these organisms were identical. In May, 1900, Strong found a dysentery organism which showed variations from the others and which has since borne his name. Kruse (1900) described a dysentery bacillus which was identical with that of Shiga. Three years later Hiss and Russell described still another type. To this the terms "bacillus Y", "Hiss" and "Hiss and Russell" have been variously applied.

Strong in Forscheimer's *Therapeutics of Internal Disease* (1917) gives a comprehensive historical review of the systematic work done with the dysentery organisms. The four types have been shown to differ from each other by agglutination and agglutinin-absorption tests, as well as by their fermentation reactions on certain carbohydrates. The Shiga type differs from the other three (Flexner-Strong-Hiss, Russell) by its lack of fermentation of a number of carbohydrates, mannitol being ordinarily the one used for differentiation. The mannitol-fermenting dysentery organisms agree in not being able to attack xylose or lactose, but differ in their behavior toward maltose and sucrose as follows:

Flexner type, maltose, acid; sucrose, negative.

Strong type, maltose, negative; sucrose, acid.

Hiss and Russell type, maltose and sucrose, negative.

Some bacteriologists regard these three as distinct species; others, as one species, with three varieties. The writer will consider them as one species for the present.

The specific name, *dysenteriae*, commonly used for these organisms, is not valid, since it was previously used by Shiga (1898) for another species. (See *Shigella dysenteriae*.) Collins (1905) used the term *Bacillus paradysenteriae*. She said, "The term paradysentery is applied to the types



which ferment mannite and differ in their agglutination reaction, but in all other respects correspond to the organism isolated by Shiga as the cause of dysentery."

There may be some question as to whether this was a proper diagnosis of the name. It will be accepted here, however, as valid. Levine (1920) used the names *Bacillus flexneri* and *Bact. flexneri* to include both the Flexner and Y types.

**Specific Diagnosis:** A short rod, conforming to the generic diagnosis. Acid is produced from mannitol, and usually from arabinose, but not from lactose or xylose, and rarely from glycerol. Fermentation of maltose, dextrin and sucrose is variable. Indol is usually produced. Litmus milk becomes slightly acid, changing to alkalinity. Is not agglutinated by "Shiga" serum. A cause of dysentery in man.

5. *Shigella alkalescens* (Andrews) Comb. nov.

Alternative: 56. *Bacterium alkalescens* (Andrews) Levine, 1920.

SYNONYMY: *Bacillus alkalescens* Andrews, 1918; *Eberthella alkalescens* (Andrews) Bergey et al, 1923.

Andrews (1918) isolated this organism from the stools of patients and named it *Bacillus alkalescens*. He described it as producing acid from glucose, maltose, mannitol and dulcitol, but not from lactose nor saccharose. Indol was formed. Litmus milk was made strongly alkaline in reaction. Levine (1920) studying twelve strains of *Shigella alkalescens* added glycerol, xylose and rhamnose to the list of carbohydrates fermented with acid. Raffinose and dextrin were not fermented.

**Specific Diagnosis:** A short rod, conforming to the generic diagnosis. It produces acid, but not gas, from maltose, xylose, mannitol, dulcitol and rhamnose. Lactose, sucrose, dextrin and salicin are not attacked. Indol is formed. Litmus milk becomes alkaline. Is not agglutinated by specific dysenteriae or paradysenteriae sera. Isolated from the stools of dysentery patients.

6. *Shigella gallinarum* (Klein) Comb. nov.

Alternative: 57. *Bacterium gallinarum* (Klein) Chester, 1897.

SYNONYMY: *Bacillus gallinarum* Klein, 1889; *Bacterium sanguinarium* Moore, 1895; *Bacillus sanguinarium* Krumwiede, Kohn, Valentine, 1918; *Bacterium jeffersoni* Hadley, Elkins, Caldwell, 1918; *Bacillus jeffersoni* (Hadley, Elkins and Caldwell) Winslow, Kligler, Rothberg, 1919; *Eberthella sanguinaria* (Moore) Bergey et al, 1923; *Eberthella jeffersonii* (Hadley) Bergey et al, 1923.

Klein (1889) described an organism isolated from a disease of hens, as a Gram-negative, non-motile, non-spore-forming bacillus. He named it *Bacillus gallinarum*. Its cultural characters were similar to those of the organisms of the colon-typhoid series.

In 1895, Moore isolated an organism from diseased hens which he called *Bacterium sanguinarium*. This organism was able to produce acid from glucose, but no gas. Lactose and sucrose were not attacked. Its morphological and cultural characters were typical of the colon-typhoid group. Hadley, Elkins and Caldwell (1918) compared this organism with a strain of Klein's organism secured by them from Europe and found them identical both culturally and agglutinatively. St. John-Brooks and Rhodes



(1923) found an organism labelled *B. gallinarum* Klein which they had secured from Král, identical culturally and agglutinatively with a strain of *B. sanguinarum* Moore received from Dr. Chas. Krumwiede. Apparently the organism under discussion should be designated by the name *Shigella* or *Bacterium gallinarum* (Klein).

The organism has been carefully studied by a number of investigators, and its cultural reactions and serological relations clearly defined. It may be differentiated from *Salmonella pullorum*, the cause of white diarrhoea in chicks, by its inability to form gas from carbohydrates. Its non-motility and ability to produce acid from mannitol place it in the Flexner section of the genus *Shigella*.

*Bacterium jeffersoni*. Hadley, Elkins and Caldwell (1918) named and described this organism which had been isolated in 1909 from a cholera-like epidemic occurring among poultry. Later work seemed to show a relation to "fowl typhoid". The organism was described as a Gram-negative, non-motile rod, which very slowly alkalined litmus milk, did not produce indol or  $H_2S$  and fermented a number of carbohydrates with acid, but no gas, including dextrose, mannose, xylose, arabinose, maltose, dextrin, dulcitol and mannitol. Lactose, raffinose, inulin, adonitol, erythritol and salicin were not attacked. Agglutination tests showed only a slight antigenic relation with fowl-typhoid strains. The homologous antigen was, likewise, only slightly agglutinated.

St. John-Brooks and Rhodes (1923), by plating a culture of *Bact. jeffersoni* Hadley and picking off smooth colonies secured a strain whose anti-serum agglutinated *Shigella gallinarum* to full titre (1-6400). Biochemically, *Bact. jeffersoni* is identical with *Shigella gallinarum*.

*Bact. jeffersoni* will be considered here as a synonym of *Shigella gallinarum* (Klein).

**Specific Diagnosis:** A rod conforming to the generic diagnosis. It ferments maltose, arabinose, xylose, dextrin, mannitol, dulcitol and rhamnose, with acid, but not gas, production. Lactose, sucrose, glycerol and salicin are not attacked. Indol is not produced. Litmus milk becomes slightly acid, followed by alkalinity. The causative agent of fowl typhoid.

7. *Shigella pfaffii* (Hadley, Elkins, Caldwell) Comb. nov.

Alternative: 58. *Bacterium pfaffii* Hadley, Elkins, Caldwell, 1918.

SYNONYMY: *Bacillus pfaffi* St. Johns-Brooks and Rhodes, 1923; *Eberthella pfaffi* (Hadley) Bergey *et al*, 1923.

Hadley, Elkins and Caldwell (1918) studied an organism secured from the Král laboratory, where it was known as the bacillus of "Kanarienvogelseuche (Pfaff)". They named it *Bacterium pfaffi*. It was found to be a Gram-negative, non-motile rod, staining well with aniline dyes, but not giving either peripheral or bipolar staining. Gelatin was not liquefied. Litmus milk was unchanged. Indol and  $H_2S$  were not formed. In its fermentation reactions it differed from *Shigella gallinarum* in producing acid from salicin and failing to attack dulcitol. It differed from *Shigella rettgeri* by its acid production from arabinose, maltose and dextrin, and its inability to attack inulin and adonitol.\*

\*St. John-Brooks and Rhodes (1923) working with an organism secured from Král and labelled *B. der Kanarienvogelseuche* (Pfaff), found it to be sluggishly motile and producing alkalinity in litmus milk.

**Specific Diagnosis:** A short rod conforming to the generic diagnosis. It produces acid, but not gas, from maltose, arabinose, xylose, dextrin, mannitol and salicin. Lactose, sucrose and dulcitol are not attacked. Indol is not formed. Litmus milk is unchanged. A cause of typhoid in birds.

8. *Shigella rettgeri* (Hadley, Elkins, Caldwell) Comb. nov.

Alternative: 59. *Bacterium rettgeri* Hadley, Elkins, Caldwell, 1918.

SYNONYMY: *Bacillus rettgeri* St. John-Brooks and Rhodes, 1923; *Eberthella rettgeri* (Hadley) Bergey *et al*, 1923.

This organism was isolated by Rettger in 1909 from an epidemic in chickens, resembling fowl cholera. Its characters were studied in detail by Hadley, Elkins and Caldwell (1918), who named it *Bacterium rettgeri*. It was a short, non-motile, Gram-negative rod, staining with aniline dyes and not exhibiting peripheral nor bipolar staining. It showed no distinctive characters on ordinary laboratory media. Litmus milk was made alkaline, becoming translucent. Indol was not formed\*. Acid was produced from glucose, mannose, xylose, adonitol, mannitol and salicin, but not from arabinose, raffinose, sucrose, lactose, maltose, dextrin, inulin, dulcitol or erythritol.

The ability to attack mannitol places the organism in the Flexner section of the genus *Shigella*. It may be differentiated from *Shigella gallinarum* by its action on litmus milk and in dulcitol and salicin.

**Specific Diagnosis:** A short rod, conforming to the generic diagnosis. It ferments xylose, mannitol and salicin with acid, but not gas, production. Lactose, maltose, sucrose, arabinose, dextrin, and dulcitol are not attacked. Litmus milk becomes alkaline, later translucent. A cause of fowl typhoid.

9. *Shigella madampensis* (Castellani) Comb. nov.

Alternative: 60. *Bacterium madampensis* (Castellani) Comb. nov.

SYNONYMY: *Bacillus madampensis* Castellani, 1912; *Bacillus dispar* Andrews, 1918 (in part); *Lankoides madampensis* (Cast.) Castellani and Chalmers, 1919; *Bacterium dispar* Levine, 1920 (in part); *Eberthella dispar* (Andrews) Bergey *et al*, 1923 (in part).

Castellani (1912) described a non-motile, Gram-negative, short rod, isolated from human stools, apparently a member of the colon-typhoid group. This organism produced acid, but not gas, from glucose, sucrose, mannitol, maltose and lactose, but did not attack dulcitol. It acidified and coagulated milk. Indol was produced. This organism was named *Bacillus madampensis*.

Andrews (1918) proposed the name *Bacillus dispar* for all lactose-fermenting members of the dysentery group. Of eleven strains which he had isolated and studied, all fermented glucose, maltose and mannitol, six fermented sucrose and two dulcitol. Indol formation was variable. Milk was acidified and eventually clotted. They resembled *Shig. alkalescens* in their relation to specific Flexner serum. Levine (1920) studied eleven strains and added sucrose, xylose and raffinose to the list of carbohydrates attacked. The series of organisms studied by Andrews and by Levine under the specie name "*dispar*" evidently included the type *madampensis* of

\* St. John-Brooks and Rhodes (1923) reported it as forming indol.

Castellani as well as another species of Castellani, which he called *ceylonensis*.

**Specific Diagnosis:** A short rod conforming to the generic diagnosis. It attacks lactose, maltose, sucrose, arabinose, xylose, glycerol, mannitol and rhamnose with acid, but not gas, production; dulcitol and salicin are not attacked. Litmus milk becomes acid and is coagulated. Indol is produced. Found in the stools and intestines of men.

10. *Shigella ceylonensis* (Castellani) Comb. nov.

Alternative: 61. *Bacterium ceylonensis* (Castellani) Comb. nov.

**SYNONYMY:** *Bacillus ceylanensis* B. Castellani, 1907; *Bacillus ceylonenses* B. Castellani, 1912; *Bacillus dispar* Andrews, 1918 (in part); *Lankoides ceylonensis* B. (Cast.) Castellani and Chalmers, 1919; *Bacterium dispar* Levine, 1920 (in part); *Eberthella dispar* (Andrews) Bergey et al 1923 (in part).

Under the name *Bacillus ceylanensis* (spelled *ceylonensis* in all subsequent descriptions) Castellani in 1907 described a rod, non-motile, Gram-negative, with cultural characters typical of members of the colon-typhoid series. It produced acid, but no gas, from glucose, sucrose, mannitol, dulcitol, maltose and lactose. Milk was acidified and clotted. The organism was pathogenic for guinea pigs and rabbits. As suggested in the discussion of *Shigella madampensis*, organisms of this type were included by Andrews in his species *Bacillus dispar*.

**Specific Diagnosis:** A short rod conforming to the generic diagnosis. It produces acid, but not gas, from lactose, maltose, sucrose, arabinose, xylose, mannitol, dulcitol and rhamnose; salicin is not attacked. Indol is produced. Milk becomes acid and coagulated. Found in the stools and intestines of humans.

11. *Shigella sonnei* (Levine) Comb. nov.

Alternative: 62. *Bacterium sonnei* Levine, 1920;

**SYNONYMY:** Group III of Sonne, 1915; *Bacillus* of Sonne, Thjotta, 1919; *Bacillus dysenteriae* Sonne, Smith, J., 1924

Sonne (1915) studied a large series of organisms from faeces and urine of dysentery patients. These organisms were non-motile and glucose and mannitol fermenters (acid only). One type (Group III), which was more abundant than all the rest, did not produce indol, made litmus milk acid, and fermented maltose, sucrose, rhamnose and usually dextrin. Agglutination reactions showed no relation to other groups of dysentery bacilli.

Thjotta (1919) confirmed Sonne's conclusions as to the existence of a serologically distinct group of dysentery organisms. He proposed three groups of dysentery bacilli: Group I, the Shiga type; Group II, Flexner, Strong and Y type; and Group III, the Group III of Sonne. He characterized the latter briefly as serologically specific, producing large, uneven colonies, fermenting mannitol, maltose and saccharose with acid, not producing indol.

Levine (1920) found the Sonne organism able to produce acid from lactose and rhamnose, but not from xylose. J. Smith (1924) studied strains of the bacillus of Sonne and recorded acid production from dextrose and

the other hexoses, from arabinose, rhamnose, lactose, sucrose, mannitol and glycerol, but not from xylose, dulcitol or sorbitol.

**Specific Diagnosis:** A short rod, conforming to the generic diagnosis. It produces acid, but not gas, from lactose, maltose, sucrose, arabinose, glycerol, mannitol and rhamnose. Xylose and dulcitol are not attacked. Indol is not produced. Litmus milk is made acid and is coagulated. Serologically distinct from the Shiga and Flexner types of dysentery organisms. Found in the faeces and urine of dysentery patients. A cause of dysentery in man.

12. *Shigella equi* (Magnusson) Comb. nov.

Alternative: 63. *Bacterium equi* (Magnusson) Weldin and Levine, 1923.

SYNONYMY: *Bacterium viscosum equi*, Magnusson, 1919.

This organism, the cause of "joint ill" in foals, was described by Magnusson (1919) under the name *Bacterium viscosum equi*. It was described as a small, oval, Gram-negative, non-motile bacillus, which made all liquid media in which it was grown slimy or ropy, although no capsules could be demonstrated. It fermented with acid production glucose, lactose, sucrose, maltose, raffinose, mannitol and galactose, but did not attack arabinose, rhamnose, adonitol and dulcitol. Gas was not formed from any carbohydrate.

The organism is evidently a valid species of the genus *Shigella*. Weldin and Levine (1923) included it under the name *Bacterium equi* in their subgenus *Eberthella*.

**Specific Diagnosis:** A short rod, conforming to the generic diagnosis. Acid, but not gas, is produced from lactose, maltose, sucrose and mannitol. It does not attack arabinose, dulcitol nor rhamnose. Colonies on solid media are mucoid; liquid media becomes slimy or ropy. The cause of "joint ill" in foals.

Genus 7. *ALCALIGENES* Castellani and Chalmers, 1919.

SYNONYMY: *Alkaligenes* Castellani and Chalmers, 1919; *Brucella* Meyer and Shaw, 1920.

This name was first used by Castellani and Chalmers (1919) to designate a genus of their tribe *Ebertheae*. They defined it as follows:

*Ebertheae* which do not ferment glucose nor lactose, and are characterized by their general lack of fermentative power and by actually increasing the alkalinity of the media. Milk is not clotted, and is rendered alkaline. Type: *Alkaligenes faecalis* (Petruschky, 1896), *emendavit* Castellani and Chalmers, 1918.\*

Their tribe *Ebertheae* possesses the general characters of the colon-typoid organisms except with regard to capsule formation, the tribe being limited to the non-capsule formers.

Weldin and Levine (1923) used the name for a subgenus of *Bacterium*, including such members of the genus as are not known to be plant pathogens and do not produce acid or gas from glucose. Bergey (1925), using the name for his sixteenth genus of the family *Bacteriaceae*, added that

\* The citation for 1918 is apparently an error, as no description for the genus can be found of this date.



acetyl-methyl-carbinol is not formed. It is felt that this character is not necessary nor hardly warranted from descriptions of the organisms available.

The lack of fermentative ability and pathogenicity sufficiently differentiates this group from the others to warrant its being recognized as a genus.

**Generic Diagnosis:** *Motile or non-motile, Gram-negative, non-spore-forming rods, Do not ferment any of the carbohydrates with acid or gas production, but often increase the alkalinity of the medium. Pathogenicity slight.*

**Type species:** *Alcaligenes faecalis* (Petruschky) Bergey *et al* 1923.

#### KEY TO SPECIES OF THE GENUS *ALCALIGENES*

- a. Gelatin not liquefied.
  - b. Capsules not formed in milk; milk not rendered slimy.
    - c. Motile.
      1. *Alcaligenes faecalis*.
    - cc. Non-motile.
      - d. Nitrates reduced to nitrites.
        2. *Alcaligenes metalcaligenes*.
      - dd. Nitrates not reduced.
        - e. Cause of Malta fever.
          3. *Alcaligenes melitensis*.
        - ee. Cause of contagious abortion in cattle.
          4. *Alcaligenes abortus*.
      - bb. Capsules formed in milk; milk rendered slimy.
        5. *Alcaligenes viscosum*.
    - aa. Gelatin liquefied.
      6. *Alcaligenes bookeri*.

#### SPECIES OF *ALCALIGENES*

1. *Alcaligenes faecalis* (Petruschky) Bergey *et al*, 1923.

Alternative: 64. *Bacterium alcaligenes* (Petruschky) Lehmann and Neumann, 1901.

**SYNONYMY:** *Bacillus faecalis alcaligenes* Petruschky, 1896; *Bacterium faecalis alcaligenes* (Petruschky) Chester, 1897; *Bacillus alcaligenes* (Petruschky) Migula, 1900; *Alcaligenes faecalis alkaligenes* (Petruschky) Castellani and Chalmers, 1919; *Alcaligenes fecalis* (Petruschky) Castellani and Chalmers, Bergey *et al*, 1923.

Petruschky (1896) first described this organism under the name *Bacillus faecalis alcaligenes*. He isolated it from typhoid stools and from spoiled beer. According to his description, the organism morphologically resembled the typhoid bacillus. It was a Gram-negative, motile rod. It did not liquefy gelatin, gave a negative indol reaction and did not produce acid or gas in sugar media. A strong alkaline reaction was produced in milk. It did not agglutinate with typhoid serum. The organism has been



isolated and studied by many subsequent investigators, but little of significant differential value has been added to its description. It has been found to be uniformly negative, both as to acid and gas production, on all carbohydrates tested.

Migula (1900) reduced the name to binomial form. Chester (1897) had placed it in the genus *Bacterium*, but had used the trinomial name. Lehmann and Neumann (1901) must, therefore, be given priority for the name *Bacterium alcaligenes*. The specific name *alcaligenes* cannot be used, however, in combination with the generic name *Alcaligenes*, (Second case, Article 55, International Rules of Botanical Nomenclature) and was, therefore, changed by Bergey *et al* (1923) to "*Alcaligenes fecalis*." The specific name should be spelled "faecalis," however, to conform to Petruschky's original spelling.

**Specific Diagnosis:** A motile rod, conforming to the generic diagnosis. Gelatin is not liquefied. Indol is not produced. Nitrates are reduced to nitrites. Litmus milk is made alkaline and is not coagulated. A slightly brownish growth is produced on potato. Not known to be pathogenic to man or experimental animals. Found in the intestines of man. Also isolated from spoiled beer.

## 2. *Alcaligenes metalcaligenes* Castellani and Chalmers, 1919.

Alternative: 65. *Bacterium metalcaligenes* (Castellani and Chalmers) Weldin and Levine, 1923.

SYNONYMY: *Alcaligenes metalcaligenes* (Adametz) Bergey *et al*, 1923.

Castellani and Chalmers (1919) described this organism as identical with their *B. faecalis alcaligenes* except for the absence of motility. They listed it as one of the causal organisms of para-enteric fever. Weldin and Levine (1923) included it in their subgenus *Alcaligenes* of the genus *Bacterium*.

Similar, but unnamed, organisms have been described by Ford (1901), Lewis (1911), Cox, Lewis and Glynn (1912), Ritchie (1914) and Pirie (1917).

**Specific Diagnosis:** A rod conforming to the generic diagnosis and similar in its morphological, cultural and biochemical properties to *Alcaligenes faecalis* except that it is non-motile. Pathogenicity, slight if any. Found in the intestines of man and animals and in milk.

## 3. *Alcaligenes melitensis* (Bruce) Bergey *et al*, 1923.

Alternative: 66. *Bacterium melitensis* (Bruce) Evans, 1918.

SYNONYMY: *Micrococcus melitensis* Bruce, 1893; *Brucella melitensis* Meyer and Shaw, 1920; *Bacillus melitensis* Khaled, 1921.

Bruce (1893) gave the name *Micrococcus melitensis* to the organism which he discovered in 1887 and concluded to be the cause of the "fièvre méditerranéenne" (now known as Malta fever). He described it as being round or oval, non-motile and Gram-negative. It developed slowly on all laboratory media at 37° and hardly at all at room temperature. Gelatin was not liquefied. Subsequent investigators have found it unable to produce acid or gas from any carbohydrate.

Evans (1918) studied six strains of varied origin. She showed it to be a Gram-negative, non-motile, non-spore-forming rod and called it *Bacterium*

*melitensis*. She found it to be biochemically and agglutinatively identical with *Bacterium abortus* Bang. Meyer and Shaw (1920) found *abortus* and *melitensis* morphologically and biochemically identical. They proposed the generic name *Brucella* for these two organisms. (See *Brucella*.) Khaled (1921) likewise found them morphologically and culturally identical, and was not able to differentiate them by biochemical methods or by agglutination reactions. From absorption experiments, *melitensis* appeared to be a substrain of *abortus*. Immunization of a monkey with a killed suspension of *Alc. abortus* protected it from subsequent infection with *Alc. melitensis*. Specific pathogenicity appears to be the only known means of differentiating the two organisms and recent work by Evans (1925) indicates an extremely close relationship with regard to this character. Perhaps they should be considered varieties of one species, under the name *melitensis*.

**Specific Diagnosis:** A non-motile rod, conforming to the generic diagnosis. Coccoid cells frequent. Gelatin is not liquefied. Nitrates are not reduced. Aerobic, or slightly micro-aerophilic. Litmus milk unchanged or slightly alkaline. Distributed in milk, particularly goat's milk. The cause of Malta fever.

4. *Alcaligenes abortus* (Bang) Bergey et al, 1923.

Alternative: 67. *Bacterium abortus* (Bang) Schmidt and Weis, 1902.

SYNONYMY: *Bacillus* of abortion (cattle) Bang, 1887; *Le bacille de* Bang, Nowak, 1908; *Abortus bacillus*, Bang, Good, 1912; *Bacillus abortus* Evans, 1915; *Bacterium abortum* (Bang) Holland, 1920; *Brucella abortus* Meyer and Shaw, 1920.

Bang (1897) described this organism as the cause of infectious abortion in cattle. It was found to be a non-motile rod growing scantily on laboratory media. Bang described it as a microaerophile. Schmidt and Weis (1902), Nowak (1908) and Good (1912) all reported partial reduction of oxygen as necessary for cultivation. Evans (1915) stated that incubation in a closed jar with *B. subtilis* favored isolation growth, but that after the strain had become accustomed to artificial cultivation, growth was abundant on all laboratory media.

Evans (1918), Meyer and Shaw (1920), Khaled (1921) and Evans (1925) compared strains of *Alcaligenes abortus* with *Alcaligenes melitensis* and found them morphologically, culturally and agglutinatively identical. (See *Alcaligenes melitensis*.) In case they were to be considered as one species, *melitensis* would be the valid specific name since it has priority over *abortus*.

**Specific Diagnosis:** A rod morphologically, culturally and agglutinatively identical with *Alcaligenes melitensis*. The cause of contagious abortion in cattle. Also causes abortion in mares, sheep, rabbits and guinea pigs.

5. *Alcaligenes viscosum* (Adametz) Comb. nov.

Alternative: 68. *Bacterium viscosum* (Adametz) Weldin and Levine, 1923.

SYNONYMY: *Bacillus lactis viscosus* Adametz, 1889; *Bacillus viscosus lactis* (Adametz) Kruse, in Flügge, 1896; *Bacterium viscosus lactis* (Adametz) Chester, 1897; *Bacterium lactis viscosum* (Adametz) Lehmann and Neumann, 1901; *Lactobacillus viscosus* (Adametz) Bergey et al, 1923; *Achromobacter viscosum* (Adametz) Bergey et al, 1925.

Adametz (1889) apparently first described this organism. He named it *Bacillus lactis viscosus*. It was isolated from slimy or ropy milk. It was a non-spore-forming, motile in young milk cultures, short rod, producing capsules in milk. Adametz considered it Gram-negative, but more resistant to decolorization than related species. Gelatin was not liquefied.

Kruse (1896) and Lehmann and Neumann (1901) described the organism as non-motile. Buchanan and Hammer (1915) did not observe motility. It should probably be considered non-motile. Investigations by various workers have shown the organism unable to attack carbohydrates either with acid or gas production.

Chester (1897) first placed the species in the genus *Bacterium*. Weldin and Levine (1923) reduced the name to binomial form and included it in their subgenus *Alcaligenes*. They used its ability to render milk slimy to differentiate it from other members of the species.

**Specific Diagnosis:** A rod conforming to the generic diagnosis. Capsules are produced in milk, rendering the milk slimy or ropy. Gelatin is not liquefied. Indol is not produced. Found in slimy or ropy milk.

6. *Alcaligenes bookeri* (Ford) Bergey *et al*, 1923.

Alternative: 69. *Bacterium bookeri* (Ford) Levine and Soppeland, 1926.

**SYNONYMY:** *Bacillus A*, Booker, 1887; *Bacillus bookeri*, Ford, 1903; *Alcaligenes bookeri* Bergey *et al*, 1925.

Booker (1887) designated as "*Bacillus A*," an organism found in cholera infantum. It was a narrow bacillus, actively motile, liquefying gelatin rapidly and blood serum slowly, and producing a definitely alkaline reaction in milk. Ford (1903) studied an organism which he had isolated from the stomach of a foundling and believed was identical with Booker's *Bacillus A*. His results showed an alkaline reaction in glucose broth, lactose and sucrose not attacked, indol not formed. He named the organism, *Bacillus bookeri*.

**Specific Diagnosis:** A motile rod\* conforming to the generic diagnosis. Gelatin is liquefied. Nitrates are not reduced. Indol is not formed. Litmus milk is peptonized with reduction of the litmus. Found in the intestines of man.

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## FUNGICIDAL ACTIVITY OF FURFURAL

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Furfural was first prepared by Döbereiner (2) in 1830. Although it has been utilized for the last forty years in the quantitative determination of pentoses and pentosans its chemical and physical properties were practically unknown until within the last few years. Until recent development in its manufacture, furfural remained too high in price to encourage laboratory experiments or commercial utilization. In 1920, Monroe (7) obtained furfural by the action of sulphuric acid on corn cobs and in 1921, LaForge (3) worked out a commercial process for its preparation from this same source. Soon after this, furfural was offered on the market at a low price through the independent research of the Miner Laboratories of Chicago (6). It was prepared by the digestion of oat hulls with steam and acid. These recent developments in the manufacture of furfural have stimulated investigations looking toward its utilization.

Furfural in its properties resembled formaldehyde on the one hand and benzaldehyde on the other. This suggested the possibility of its use as a fungicide. Such studies were undertaken at Iowa State College through a fellowship of the Crop Protection Institute in January, 1924, and the results of some of the laboratory toxicity studies, together with some of the substantiating field data are presented in this paper. The termination of the project after one year precludes the presentation of all of the results obtained due to lack of adequate confirmation. The studies presented include the comparative effects of furfural and formaldehyde on the germination of seeds and sclerotia, and the influence of these same aldehydes and related compounds on the germination of spores of *Puccinia coronata* Cda. *holci*, *Sphacelotheca sorghi* (Lk) C1 and *Ustilago hordei* (Pers) K & S.

### Laboratory Studies

#### The Comparative Toxicity of Six Aldehydes.

The comparative toxicity of furfural and other aldehydes to fungus spores was studied, using the van Tieghem cell. Since all germination tests were at room temperature, little difficulty was experienced with breaking of the hanging drop due to excessive condensation of moisture on the cover slip. The glassware composing the cell was never used twice without being taken apart and washed in xylol, dried, boiled in alkali, in cleaning solution, and in several changes of distilled water. It was dipped in alcohol and wiped dry and sterilized in an oven for one hour.

Six aldehydes: formaldehyde, acetaldehyde, propylaldehyde, butylaldehyde, furfuraldehyde and benzaldehyde were used in these experiments. Stock solutions were made up of M/10 concentration of all these except benzaldehyde, for which an M/50 solution was used. These were stored in the dark in pyrex glassware and care exercised to keep the bottles stoppered.

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Three organisms, *Puccinia coronata holci*, *Ustilago hordei*, and *Sphacelotheca sorghi*, were used to measure toxicity. These were uniformly variable, germinated readily in distilled water in a short period of time at room temperature and were easily obtained. The influence which a variation in the number of spores in a hanging drop has on toxicity was not observed in these tests, but an effort was made to introduce about the same number of spores in each drop. Ten trials were made on different dates, using three replications in each trial of a given species.

Criteria which may be used as an indication of toxicity in a test of this kind are: (1) percent of spores germinating and (2) average length of germ tubes after allowing the culture to incubate for a definite length of time under specified conditions. The first is the most widely used. Because of the small size of the spores of *Sphacelotheca sorghi* and *Ustilago hordei*, and because they form a dense film at the base of the hanging drop an accurate count of germination was not practical. The response of these spores to the aldehydes was based on a careful comparison with check cultures in distilled water. The rapidity of germination was determined by intermittent observation for the first 24 hours. Toxicity to the uredospores of *Puccinia coronata holci* was determined by examining the cultures at regular intervals and accurately counting the percent of germination and measuring a representative number of germ tubes from several fields in each drop.

The effect of six aldehydes on the germination of spores of *Sphacelotheca sorghi*, *Ustilago hordei*, and *Puccinia coronata holci*, after a 24 hour incubation period is shown in Table I. There is a certain degree of constancy in the molar concentration of the different aldehydes which inhibited spore germination. A concentration of 0.005 M entirely prevented the germination of the spores of at least one of the three organisms tested, and noticeably retarded germination of the others. The 0.0025 M concentration usually retarded germination, while the 0.001 M solution usually had no effect.

There are some variations in the toxic concentration of the aldehydes which indicate specific action.

Taylor (10) has demonstrated the specificity of several organic acids on four different groups of wound inhabiting bacteria, but the writer is unaware of any work on the specificity of organic compounds to fungi. The only indication of specific action was the tolerance of *Ustilago hordei* to formaldehyde and its intolerance to furfural in comparison with the tolerance of *Puccinia coronata holci* to furfural and extreme sensitiveness to formaldehyde. In the case of *Ustilago hordei*, a solution of furfural was more inhibitory to germination than a solution of formaldehyde possessing two and a half times its molar concentration. With *Puccinia coronata holci* a formaldehyde solution produced as great a toxic action as a furfural solution ten times as concentrated. Except for this sensitivity of *P. coronata holci* and the slightly greater tolerance of *Ustilago hordei* to formaldehyde, the molar concentration of the aldehydes, which inhibited spore germination, was quite uniform.

Because of the specificity of action of the different aldehydes the most toxic could not be definitely determined. However, these limited experiments indicated that benzaldehyde, formaldehyde and butylaldehyde were most toxic, followed by furfuraldehyde and propylaldehyde, with acetaldehyde the least.

TABLE I. COMPARATIVE TOXICITY OF SIX ALDEHYDES TO SPORES OF *SPHACELOTHECA SORGHII*, *USTILAGO HORDEI* AND *PUCCINIA CORONATA HOLCI*.

Molecular concentration of solution tested	Sphacelotheca sorghi						Ustilago hordei						Puccinia coronata holci					
	Formaldehyde	Acetaldehyde	Propylaldehyde	Butylaldehyde	Furfuraldehyde	Benzaldehyde	Formaldehyde	Acetaldehyde	Propylaldehyde	Butylaldehyde	Furfuraldehyde	Benzaldehyde	Formaldehyde	Acetaldehyde	Propylaldehyde	Butylaldehyde	Furfuraldehyde	Benzaldehyde
0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.0075	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.005	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.0025	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.001	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.00075	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.0005	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.00025	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

0 = No germination.

tr. = Trace, an occasional spore germinating by a weak promycelium.

± = Retarded germination.

+ = Normal germination as in distilled water.



### Correlation of Toxic Action on Spore Germination and Its Subsequent Growth

The toxic effect of the aldehydes on the process of germination of *Puccinia coronata holci* was studied in more detail. The culture in distilled water was put under a microscope and the time required for the first indication of germination recorded. This occurred in from 70 to 80 minutes. The other cultures were examined after a lapse of an equal period of time and in only one case, the 0.0001 M formaldehyde, where the process of germination was at a stage similar to that in the check, was there any sign of germination. All of the tests could not be made simultaneously so that the percent of germination and the average length of germ tube in the check cultures of each series of tests was considered as 100 of the aldehyde cultures figured accordingly. The figures are given in Table II.

The cultures were examined after germination had first been observed in the checks, and after two, four, eight and twenty-four hour periods. The length of the germ tubes in the eight and twenty-four hour cultures, all 750 $\mu$  or more, made accurate counting and measurement of the spores and germ tubes impractical. The only information of value obtained from these examinations was that no perceptible difference between the eight and twenty-four hour cultures occurred and that the spores never fully recovered from the initial retarding effects of the aldehydes. The two hour examination showed that all of the aldehydes produced marked retarding effects near the concentration inhibiting spore germination as indicated both by the percent of germination and by the average length of germ tube. Formaldehyde, as a rule, appeared to reduce the length of germ tube more than the number of spores germinating, while with the others the reverse was quite uniformly the case. In the four hour examination the retarding effect produced by aldehydes near the concentration inhibiting germination was further brought out by the starting of germination in 0.005 M furfural and propylaldehyde and in the 0.0025 M butylaldehyde. The lower figures in the 0.0005 M formaldehyde showed that germination in this solution did not keep pace with that of the checks. The eight hour examination revealed the beginning of germination during the four to eight hour period in the 0.00075 M formaldehyde solution. It also showed a general tendency for recovery as indicated by a narrowing of the difference in germ tube length between the check cultures and those of the solutions.

### Toxicity of Metallic Furoate Salts

The furfural molecule is peculiarly constructed in that besides having the aldehyde group it has in the furyl radical an unsaturated oxygen atom. It is possible that some of the chemical reactions of furfural take place by means of this unsaturated oxygen. It appeared desirable, therefore, in a study of the toxic properties of furfural to determine the role played by the furyl radical.

The means used to determine this was a comparison of the toxicity of several metallic furoate salts. These were prepared expressly for this purpose through the kindness of Dr. J. P. Trickey of the Miner Laboratories, Chicago, Illinois.

In Table III the results obtained in testing the toxicity of five metallic furoate salts to spores of *Sphacelotheca sorghi* are given. The van Tieghem cell method described by Clark (1) was used. Apparently the unsaturated oxygen in the furyl radical was not an important factor in the toxicity

of furfural, as the calcium and ferric furoates were only slightly toxic. The calcium furoate molecule contains two furyl radicals, while furfural contains only one, consequently in equimolar solutions of calcium furoate and furfural there are twice as many furyl radicals in the former as there are in the latter. The retarding effects produced by the 0.004 M calcium furoate and the 0.0025 M furfural solutions were approximately the same. That is, a solution of furfural was as toxic as one of calcium furoate containing three times as many furyl radicals. This suggests that the aldehyde group was a more important factor in toxicity than the furyl radical.

The order of toxicity of the metallic salts corresponds with the toxicity of the metals they contain. Mercuric furoate was the most toxic. Cupric and nickle furoates were of intermediate toxicity and calcium and ferric furoates were the least toxic.

TABLE III. TOXICITY OF METALLIC FUROATE SALTS TO SPORES OF *SPHACELOTHECA SORGHII*.

Material tested	Percent concentration	Molar concentration	Germination
Calcium furoate	1.0	.04	None
" "	0.1	.004	Abundant but retarded
" "	0.01	.0004	Normal
Ferric furoate	0.25	.007	None
" "	.025	.0007	Normal
Nickle furoate	0.1	.004	None
" "	0.01	.0004	Abundant but retarded
" "	0.001	.00004	Normal
Cupric furoate	0.01	.004	None
" "	0.001	.0004	Abundant but retarded
" "	0.0001	.00005	Normal
Mercuric furoate	0.05	.001	None
" "	0.005	.0001	Greatly retarded
" "	0.0005	.00001	Normal
Check water			Normal
Furfural	.05	.005	None
" "	.025	.0025	Retarded
" "	.01	.001	Normal

#### The Toxic Action of Furfural on the Sclerotia of *Rhizoctonia solani* on Potatoes

The comparative effectiveness of furfural and formaldehyde solutions in the control of the *Rhizoctonia* disease of potatoes was studied in the laboratory following the methods used by Melhus, Gilman and Kendrick (5). Potato tubers infected with sclerotia of *Rhizoctonia solani* were dipped into the solution to be tested. The sclerotia were removed from them after treatment and plated on potato agar. These were incubated for 36 to 48 hours and the number of sclerotia germinating determined. Trials were made at each temperature and concentration with 20 sclerotia per plate.

Table IV shows that raising the temperature of the solution accelerated the toxic action of furfural. With furfural solutions of equal concentration the short treatments at 50° to 60° C. were invariably more toxic than the long time soaks at room temperature. Sclerotia soaked in one percent furfural for two minutes at 55° C. to 60° C. showed only two percent and one percent germination, respectively. Although the two percent furfural solution was much more toxic at room temperature than the one percent solution, raising the temperature also markedly increased its toxicity.



Sclerotia soaked at 20° C. for 15 minutes showed 85 percent, those soaked for 30 minutes, 75 percent, and those soaked for 60 minutes 10 percent germination. Only one to two percent of the sclerotia soaked in two percent furfural at 50°, 55° and 60° C. for two minutes germinated. In general these results are in accord with the data presented by Raeder, Hungerford and Chapman (8). They found that increasing the time and temperature accelerated the toxic action of furfural, but that it was not as toxic to the sclerotia of *Rhizoctonia* as formaldehyde. Their results suggest further that *Rhizoctonia* sclerotia grown in Idaho are more resistant not only to furfural, but also to other disinfectants.

TABLE IV. EFFECT OF RAISING THE TEMPERATURE OF FURFURAL SOLUTIONS ON SCLEROTIAL GERMINATION OF *RHIZOCTONIA SOLANI*.

Treatment	Temperature Degrees C.	Length of treat- ment—minutes	Percent germination
Check, no treatment			100
Furfural 1%	20	60	68
" 2%	20	15	85
" 2%	20	30	75
" 2%	20	60	10
" 1%	50	2	30
" 1%	55	2	2
" 1%	60	2	1
" 2%	50	2	1
" 2%	55	2	2
" 2%	60	2	1

Melhus, Gilman and Kendrick (5) found that no known treatment killed all the sclerotia, but considered a treatment as sufficiently toxic which would reduce the germination to two or three percent. Accordingly, the two percent furfural treatment at 50° C. for two minutes appeared to be the minimum strength which met these requirements.

The effect of covering on the toxicity of furfural was determined by treating the tubers in two percent furfural at 50° C. for two minutes and covering in various ways. Dry burlap sacks, burlap sacks wet in two percent furfural, stone crocks inverted over the potatoes on a cement floor, and large glass jars with closely fitting tops were used as covers. Preliminary trials indicated that the type of cover had little influence on the effectiveness of the treatment. Consequently the method of using burlap sacks wet in the solution used for treating the tubers was adopted as being the most practical cover.

TABLE V. EFFECT OF LENGTH OF COVERING AFTER TREATMENT OF POTATOES WITH TWO PERCENT FURFURAL AT 50° C. FOR TWO MINUTES ON THE GERMINATION OF THE SCLEROTIA OF *RHIZOCTONIA SOLANI*.

Duration of cover	Number of sclerotia	Percent germination
Check, no treatment	100	93
Uncovered	100	5
1 hour	400	2.3
5 hours	400	0.5
12 hours	300	0.7

Further trials, as shown in Table V, using wet burlap as a cover, indicated that periods of cover up to five hours increased the effectiveness of the treatment, but that nothing was gained by a longer period of cover.

The effect of the different treatments, used in the 1925 field trials on sclerotia, was tested in the laboratory. Twenty tubers were selected at random from the treated lots and the sclerotia plated out for germination tests. The results of these tests are given in Table VI. The 1-120 formaldehyde and the three and two percent furfural treatments, both one and five hour cover, were quite effective. The one percent furfural treatments were more effective than the hot 1-1000 mercuric chloride treatment, but all of these were unsatisfactory. The five hour cover, in all cases, resulted in a smaller percent of sclerotia surviving than in the one hour cover treatments. This difference, however, was small.

TABLE VI. VIABILITY OF THE SCLEROTIA OF *RHIZOCTONIA SOLANI* IN THE SEED LOTS TREATED FOR FIELD PLANTING IN 1925.

Treatment	Length of cover in hours	Number of sclerotia	Percent germination
1. Untreated check	0	200	98.0
2. Formaldehyde 1-120 2 min. at 50° C.	3	200	3.0
3. Mercuric chloride 1-1000 2 min. at 50° C.	5	200	20.0
4. Furfural 3 percent 2 min. at 50° C.	1	200	6.0
5. Furfural 3 percent 2 min. at 50° C.	5	200	4.5
6. Furfural 2 percent 2 min. at 50° C.	1	200	5.0
7. Furfural 2 percent 2 min. at 50° C.	5	200	3.0
8. Furfural 1 percent 2 min. at 50° C.	1	200	17.5
9. Furfural 1 percent 2 min. at 50° C.	5	200	13.0

#### The Relation of Moisture to the Toxic Action of Furfural

Consideration was given to the effect of moisture on the toxic action of the vapor of furfural, formaldehyde and related compounds. Three museum jars were provided for each substance tested and an unstoppered bottle containing 30 c.c. of the chemical was placed in each jar. In one jar was placed 150 kernels of dry corn, in another a like number with a small beaker of water to maintain a saturated atmosphere, and in the third, kernels which had been presoaked for four hours. At the end of 24, 48, 72 and 120 hour periods 10 kernels were removed from each jar and placed in petri dishes on moist filter paper to germinate. The results are presented in Table VII.

Table VII shows that the action of furfural and formaldehyde relative to moisture is not the same. This difference in action was most pronounced where the presoaked and dry seed were subjected to fumes from the concentrated solutions. At the end of five days presoaked corn exposed to the fumes of 95 percent furfural was dead, while the germination of that subjected to the fumes of 40 percent formaldehyde was only slightly retarded. With dry corn the reverse was true. Seed subjected for five days to the vapor from 95 percent furfural germinated normally, while that exposed to vapor from 40 percent formaldehyde was dead. With a saturated atmosphere injury to dry corn was increased with 95 percent furfural, while it was decreased with 40 percent formaldehyde. The actions of the five percent solutions of furfural and formaldehyde were similar to those of the concentrated solutions, but greatly moderated.

Furfuralcohol in no case produced injurious effects on the germination of corn. This indicated that under the conditions of the experiment it was much less toxic than furfuraldehyde and that the furyl radical did not play an important role in toxicity.

TABLE VII. THE EFFECT OF ATMOSPHERIC MOISTURE ON THE TOXICITY OF DIFFERENT VAPORS TO SEED CORN GERMINATION.

Chemical		State of seed	Atmospheric humidity	Germination			
				24 hrs.	48 hrs.	72 hrs.	120 hrs.
Furfural	95%	Presoaked in water	Not saturated	N	R	R+	D
Formaldehyde	40%	Presoaked	" "	N	N+	N±	N—
Furfural	5%	Presoaked	" "	N	N	N+	R
Formaldehyde	5%	Presoaked	" "	N	N	N	N
Furylalcohol		Presoaked	" "	N	N	N	N
Furylalcohol	5%	Presoaked	" "	N	N	N	N
Methyl alcohol	95%	Presoaked	" "	N	R	D	D
Check		Presoaked in water	" "	N	N	N	N
Furfural	95%	Dry	Saturated	N	N	N±	N±
Formaldehyde	40%	"	"	N	N	N	N
Furfural	5%	"	"	N	N	N	N
Formaldehyde	5%	"	"	N	N	N	N
Furylalcohol		"	"	N	N	N	N
Furylalcohol	5%	"	"	N	N	N	N
Methyl alcohol		"	"	N	N	R	R
Check		"	"	N	N	N	N
Furfural	95%	"	Not saturated	N	N	N	N
Formaldehyde	40%	"	" "	N	R+	R+	D
Furfural	5%	"	" "	N	N	N	N
Formaldehyde	5%	"	" "	N	N	N	N
Furylalcohol		"	" "	N	N	N	N
Furylalcohol	5%	"	" "	N	N	N	N
Methyl alcohol		"	" "	N	R+	R+	D
Check		"	" "	N	N	N	N

R = Retarded N = Normal D = Dead + = Excessive ± = Slightly less

#### The Comparative Cumulative Injury of Furfural and Formaldehyde to the Germination of Wheat

Miss Hurd (4) made an intensive study of the injury to the germination of wheat seed following formaldehyde disinfection. She found that no injury was produced if the treated seed was planted immediately in damp soil or if it was stored damp until planted. The injury was cumulative, i. e., increased with length of storage after treatment. Miss Hurd concluded that the injury was due to the formation of paraformaldehyde on the seed, which slowly volatilized, thus subjecting the seed to a prolonged formaldehyde treatment.

The comparative initial and cumulative injury of furfural and formaldehyde was determined by soaking wheat seed in solutions of 95 percent furfural and 40 percent formaldehyde for varying lengths of time and planting periodically. Two hundred seeds of each treatment were planted in clean sand on a greenhouse bench immediately after treatment before the seed had dried, on the same day of treatment after the seed had dried, and after the treated seed had been stored 4, 22 and 45 days. The percent germinating seed was counted after eight days as shown in Table VIII.

The data in Table VIII show that solutions of formaldehyde were more injurious to wheat germination than were those of furfural. The only treatments which undoubtedly injured germination when the seed was planted immediately after treatment, while wet, were the one, two and six

hour soaks in 0.5 percent formaldehyde, and the six hour soak in 0.33 percent formaldehyde. The only effect produced by drying the treated seed, or in storing it four days before planting was an increase in the injurious effects of those treatments, which were harmful to the seed when planted wet. Storage of the treated seed for 22 and 45 days only served to aggravate the injury caused by all of the 0.33 and 0.5 percent formaldehyde treatments. The 0.1 percent formaldehyde treatments as well as all of the furfural treatments apparently were not detrimental to the germination of wheat seed even when stored for 45 days after treatment. Injury by the stronger formaldehyde solutions was cumulative, while furfural injury was not.

## Field Experiments

### The Control of Cereal Smuts

Field experiments for the control of cereal smuts covered a period of two years. During the first year efforts were chiefly directed toward determining the possibility of substituting solutions of furfural for those of formaldehyde. The second year's trials were chiefly concerned in verifying the outstanding results of the previous year and in ascertaining the toxic properties of various furfural dust derivatives.

*Source of Seed.* Only naturally infected seed was used in these experiments. Lots of wheat, variety Prelude; oats, variety White Tartar, and barley, variety Manchuria, were obtained from Mr. H. A. Rodenhiser, in charge of the Crop Protection Institute's Cereal treatment project, University Farm, St. Paul, Minnesota, and winter wheat, variety Kanred, from Mr. J. J. Wilson, Muscatine, Iowa.

*Methods Used.* The seed was treated by using methods approximating as closely as possible those used in commercial practice. The dust treat-

TABLE VIII. CUMULATIVE INJURY OF FURFURAL AND FORMALDEHYDE ON WHEAT SEED GERMINATION.

Treatment	Duration of dip	Percent germination of seed after				
		0 da.		4 da.	22 da.	45 da.
		Wet	Dry			
Check water		93.0	90.5	93.0	89.0	72.0
Furfural .1 %	1 hour	90.0	89.5	93.0	89.5	66.5
Furfural .33	1 hour	93.5	90.5	89.5	91.5	58.5
Furfural .5	1 hour	91.0	84.5	92.0	90.5	60.5
Formaldehyde .1	1 hour	81.5	86.5	92.5	90.5	59.5
Formaldehyde .33	1 hour	86.5	80.5	81.5	65.0	26.0
Formaldehyde .5	1 hour	79.5	67.0	67.5	57.5	22.0
Furfural .1	2 hours	91.0	90.0	94.0	91.5	76.0
Furfural .33	2 hours	88.0	90.0	89.0	88.0	66.0
Furfural .5	2 hours	91.0	88.5	90.0	88.0	63.5
Formaldehyde .1	2 hours	88.0	91.0	92.5	92.0	75.5
Formaldehyde .33	2 hours	88.5	78.0	87.0	83.0	44.5
Formaldehyde .5	2 hours	79.0	55.5	66.0	53.0	24.5
Furfural .1	6 hours	89.0	92.0	89.0	86.0	71.5
Furfural .33	6 hours	89.5	90.5	89.5	76.5	63.0
Furfural .5	6 hours	87.0	86.0	87.5	84.5	61.5
Formaldehyde .1	6 hours	87.5	92.0	91.0	83.0	66.5
Formaldehyde .33	6 hours	73.0	78.0	62.5	50.0	44.5
Formaldehyde .5	6 hours	49.5	56.5	35.5	18.0	10.5



ments were applied by placing the seed and dust in large manila envelopes and shaking until the dust was evenly distributed over the seed. For the soak treatments the seed was placed in muslin bags and dipped into the solutions the required length of time. For the sprinkle treatments the seed was placed in a jar, one-tenth its volume (40 gal. to 50 bu.) of solution sprinkled over it, and stirred until evenly distributed. In those treatments demanding covering the seed was placed in small crocks covered with a pane of glass. After treatment the seed was spread out until thoroughly dry and then placed in individual packages for row planting. The dust treatments were applied from one to three weeks and the liquid treatments from two to three days before planting.

Disease control was the primary object of these experiments. Consequently each treatment consisted of a single row replicated four times.

The effect on germination was determined by planting 200 seeds of each treatment in sand on a greenhouse bench and counting the number germinating after seven days.

The determinations for the percent of bunt in 1924 were made by clipping 100 heads of each row and in 1925 by clipping all the heads in each row. The percent of loose smut of wheat was determined in 1924 by counting the number of heads affected with this disease in 100 heads taken from each row. The percent of covered smut of barley and of the oat smuts was found by counting all the heads in each row and the number of heads attacked by the smut organisms.

Table IX presents the data obtained on the effects of the various treatments on bunt and loose smut of wheat, covered smut of barley, and the smuts of oats.

#### TILLELIA LAEVIS ON WHEAT

The low percent of bunt obtained in the check plots of the 1924 spring wheat series renders an interpretation of the results difficult. Two things stand out pre-eminently: first, despite the small amount of bunt in the check plots none of the furfural derivatives satisfactorily controlled the disease; second, the high percent of bunt obtained with all of the furfural liquid treatments was in contrast with low percent in the formaldehyde treatments.

Copper carbonate was the only dust treatment which gave satisfactory control of bunt. The five furfural dust derivatives used in this series gave varying degrees of reduction, but none completely eliminated it. An increase in the quantity of dust did not proportionately reduce bunt as the eight ounce furfuramide treatment had more smutted heads than either the two or four ounce treatments. The difference between the percent of smut in the two, four and eight ounce furfurine treatments was negligible. The eight ounce furoin treatment had only slightly less than the checks. The two ounce furoic and furfuraerylic acid treatments had more smut than the two ounce furfurine treatment and both of these chemicals were somewhat injurious to seed germination.

The furfural liquid treatments of wheat in 1924 yielded a surprisingly great increase in percent of bunt over the untreated check. In the sprinkle treatments there was no apparent correlation between the concentration of the solution used or length of cover and percent of bunt. The 0.75 percent furfural treatment gave the most and the 0.5 percent treatment gave



TABLE IX. A COMPARATIVE STUDY OF SMUT CONTROL ON CEREALS.

Treatment	Percent						Percent Ustilago hordel	Percent Ustilago levis and U. avenae	
	Treatment		Tilletia tritici					Oats	
			Oz. per bu.	Parent fungus	Spring wheat				
	1924	1925			1924	1925		1924	1925
1. Copper carbonate	2.00			0.00		0.00			8.4
2. "	3.00						0.00	0.5	
3. "	2.00			0.00					
4. Furfuramide	1.00			1.5		4.3	0.21	1.75	14.0
5. "	2.00					5.2			
6. "	2.66			0.5		6.8	0.16	1.25	
7. "	4.00			2.0		6.2	0.00	1.25	
8. "	8.00					4.8			
9. Furfurine	1.00			1.0		5.8	0.42	2.75	
10. "	2.00			0.8		2.0	0.21	1.25	
11. "	4.00			0.8			0.16	0.5	
12. "	8.00			3.0			0.21	1.0	
13. Furoin	2.00			1.3			0.21	1.0	
14. Furoic acid	2.00			2.0			0.30	1.0	
15. Furfuracrylic acid	2.00								3.3
16. Mercuric chloride	2.00								3.2
17. "	5.33								13.6
18. No treatment	1.00								
19. Copper stearate	2.00			1.8		3.9	0.75	3.0	
20. "	2.00					2.97			
21. Mercuric furoate	2.00								11.6
22. Ferric furoate	2.00								10.4
23. Calcium furoate	2.00					0.0			15.4
24. Nickel furoate	2.00					0.2			16.1
25. Cupric furoate	2.00					0.0			4.2
26. Mercuric furoate + calcium carbonate (1:4)	2.00					0.0			9.1
27. Ferric furoate + calcium carbonate (1:4)	4.00					0.0			10.2
28. Calcium furoate + calcium carbonate (1:4)	4.00					0.0			14.1
29. Nickel furoate + calcium carbonate (1:4)	4.00					0.2			14.2
30. "	4.00					0.0			11.1

TABLE IX—(Continued).

[illegible]

the least smut, 33.5 percent and 15.3 percent, respectively. The strongest concentration averaged 23.6 percent bunt, while the weakest averaged 24.8 percent. Although the five furfural treatments, dried immediately after treatment, averaged 20.1 percent bunt, while those covered for three and twelve hours averaged 24.9 and 25.5 percent, respectively, these differences are not significant as the individual variations were large.

The formaldehyde 1-320 or 0.03 percent treatment ordinarily recommended gave complete control of bunt.

Seed soaked in furfural solutions did not show as great an increase in bunt as the sprinkle treatments. There was a marked increase, however, which, as with the sprinkle treatments, possessed little correlation with either strength of treatment or duration of soak. In all cases the two hour soak treatments had more bunt than the corresponding six hour treatments, but in two of the three instances this difference was negligible. In the two hour soak the strongest (0.5 percent) and the weakest (0.05 percent) furfural solutions gave approximately the same amount of smut, and in the six hour soak the greatest amount of smut was present in the 0.5 percent solution.

The increase in percent of bunt produced by the furfural solutions was difficult to explain. No evidence of stimulation by furfural had ever been observed in the numerous spore germination tests conducted in the laboratory using *Tilletia laevis*. However, no water treatments had been included in the spring wheat series of 1924. In all subsequent tests these were incorporated.

The check untreated plots of the 1925 spring wheat series produced even less bunt than did the 1924 series. Consequently all the positive control results were insignificant. The dust treatments which did not give perfect control may be regarded as unsatisfactory and include the one ounce furfuramide, the two ounce calcium furoate, and the four ounce calcium furoate plus lime treatments.

The sprinkle treatments gave a marked increase in percent of bunt over the checks as in the previous year. Here again there was no correlation between concentration of solution or length of cover and percent of smut. However, the water sprinkle checks showed this same increase. The average percent of smut in the 15 furfural treatments was 5.0, while in the three water treatments it was 6.4. In consideration of the great variation of bunt in the furfural treatments, from 0.8 to 10.2 percent, this difference is insignificant.

The 1924-25 winter wheat series differed in some respects from the spring wheat series. The standard treatments, formaldehyde (1-320) sprinkle, and the two ounce copper carbonate, were the only ones which gave satisfactory control. The furfural derivatives, furfuramide and furfurine, did not reduce smut as in the two spring wheat series. Except in one instance, seed treated with these substances had slightly more smut than the untreated. The furfural sprinkle treatments did not increase the percent of smut as in the spring wheat treatments. The three furfural sprinkle treatments averaged 4.0 percent smut while the check untreated had 3.9 percent. The water sprinkle check had 14.5 percent bunt, which was higher than any other treatment. In contrast with the irregular results obtained in the furfural soak treatments with spring wheat was the steady reduction in smut obtained with an increase in concentration of the

solution in the winter wheat. The 0.5 percent furfural soak for six hours, which quite severely injured the stand, gave only 0.5 percent smut, while the 0.1 percent furfural gave 7.3 percent smut and the 0.05 percent furfural gave 10.0 percent smut. The water soak treatment had only 4.0 percent smut.

#### USTILAGO TRITICI ON WHEAT

The spring wheat of the 1924 series contained a considerable quantity of loose smut. Since the collection of data on the effect of the various treatments on loose smut required little additional labor, notes were taken on it. As was probably to be expected, treatments with furfural and formaldehyde, although strong enough to severely injure the seed, produced no appreciable effect on the percent of loose smut.

#### USTILAGO HORDEI ON BARLEY

The effect of furfural on covered smut of barley was tried only in 1924 because of the difficulty in getting seed which would produce a heavily smutted crop. In 1924 the untreated checks averaged 0.69 percent smut. Because of this low percent in the checks, too great significance should not be attached to the results obtained. All of the dust treatments reduced the percent of smut and two of them, the eight ounce copper carbonate and furfuramide treatments, completely eliminated it. In the furfural sprinkle treatments there was a consistent increase in percent of smut from 0.6 percent in the 0.33 percent treatment to 1.5 percent in the 2.0 percent treatment. In the furfural soak treatments no consistency existed between concentration and percent of smut. The formaldehyde sprinkle and both the 0.5 percent formaldehyde soak treatments gave complete control of covered smut of barley.

#### USTILAGO AVENAE AND USTILAGO LAEVIS ON OATS

The effect of furfural seed treatments on oat smut was tried in 1924 and in 1925. There was a low percentage of smut in the check plots which made the first year's trials less conclusive. These trials showed, however, that furfural dust derivatives and the furfural sprinkle and soak treatments would not control oat smut. The dust derivatives, while in all cases reducing the percent of smut, in no case gave satisfactory control. The furfural sprinkle and soak treatments gave no evidence of any effect on the amount of smut. All of the formaldehyde soak treatments completely controlled oat smut except the 0.05 percent for two hours.

The 1925 untreated plots, oat smut series, averaged 13.3 percent smut. Those furfural dust derivatives which were tried in the 1924 series and gave no evidence of control as well as the furfural soak treatments were not repeated. Some sprinkle treatments were repeated in order to determine if conditions influencing the increase of bunt in wheat were not also common to oats. A series of metallic furoate salts were tested. Also, an attempt was made to verify the results obtained by Thomas (11) with mercuric chloride dust.

Five sprinkle treatments were used. Formaldehyde (1-320) gave almost complete control of oat smut. The water sprinkle treatment had 18 percent smut, the highest in the whole series. The two percent furfural sprinkle treatment with three hour cover reduced smut to six percent, while the

one and 0.33 percent treatments had nearly the same amount as the untreated checks.

The metallic furoate salts differed greatly in their efficiency. Nickle furoate was the most toxic and cupric furoate a close second. Mercuric furoate is quite insoluble in water and probably for this reason was not as toxic as the nickle and cupric salts. Calcium and ferric furoates and calcium carbonate did not reduce the amount of oat smut. Three ounces of copper carbonate reduced oat smut, but not significantly, while two ounces of copper stearate was even less efficient.

A series of treatments with mercuric chloride as the base was used. Finely ground mercuric chloride was tested alone at the rate of two and 5.33 ounce quantities. A three ounce treatment of a mixture consisting of two parts mercuric chloride and one part copper carbonate as recommended by Thomas (11) was also used, as was one consisting of two parts mercuric chloride and one part Sil O'Cil<sup>1</sup>. Also a mixture of one part furfuralamide and two parts mercuric chloride was used in varying quantities. All of the mercuric chloride treatments gave a considerable reduction in the amount of smut, but none controlled it as did the formaldehyde treatments. The two and 5.66 ounce mercuric chloride treatments and the three ounce mixtures of mercuric chloride and copper carbonate and of mercuric chloride and Sil O'Cil gave almost the same degree of smut control, ranging from 2.9 percent to 3.3 percent. A mixture of furfuralamide and mercuric chloride react to form a resin. This apparently interfered to some extent with the toxic properties as the percent of smut was higher in all cases than where the mercuric chloride alone was used. There was not a proportionate decrease for an increase in quantity of material used as the nine ounce treatment was little better than the three ounce treatment.

#### THE TOXIC ACTION OF SEED DISINFECTANTS

Table X presents the results obtained in germination tests following the treatment of the seed planted in 1924 and 1925.

The dust treatments with the exception of furfuralacrylic acid and those in which mercuric chloride was an ingredient were not injurious to germination. In the 1924 spring wheat series where the seed germinated poorly the dust treatments apparently were beneficial. The untreated seed averaged 71.0 percent germination, while the nine dust treatments averaged 84.9 percent. The germination of the oats and barley in 1924 and of spring wheat in 1925 was comparatively good so that any beneficial results of treatment were not apparent.

All of the mercuric chloride treatments were injurious to germination. Injury was more severe to wheat than to oats. While the mercuric chloride treatments, in most cases, caused reduction in germination, injury was much more apparent through a retardation in germination and a decrease in vigor. Plants from seed treated with mercuric chloride would emerge two to three days late and would be distorted. This retardation and distortion was proportional to the amount of mercuric chloride used. Mixing the mercuric chloride with a filler as copper carbonate, Sil O'Cil or furfuralamide served to ameliorate this injurious action to some extent.

The furfural and formaldehyde liquid treatments had no stimulatory effect on germination. The weaker treatments had apparently no effect,

<sup>1</sup> Sil O'Cil is an inert, light weight silicious substance, a diatomaceous earth.



while the stronger ones injured germination in all cases. Injury was proportional to the concentration of the solution, the duration of soak, and the duration of cover. Formaldehyde was more injurious to germination than an equal concentration of furfural used under similar conditions. Formaldehyde was more harmful to wheat than to oats or barley and was more harmful to oats than to barley. Wheat and oats were about equally susceptible to furfural injury, while barley was more resistant.

#### The Control of Black Scurf, *Rhizoctonia solani*, of Potatoes in 1924 and 1925

The effectiveness of furfural solutions for the control of the black scurf disease of potatoes was tested in the field. In 1924, two early varieties, Early Ohio and Irish Cobbler, and one late variety, Rural New Yorker, were used. In 1925 only one variety, Early Ohio, was tested. The land on which the potatoes were grown in 1924 was a sandy loam, low in organic matter, and had grown three successive crops of potatoes. The land used in 1925 was a sandy loam, well supplied with organic matter, and had not grown potatoes for at least ten years.

The treatments used in the field were based on the information obtained from the laboratory experiments. In 1924 four check treatments were used: (1) clean seed treated with 1-120 formaldehyde for two minutes at 50° C.; (2) clean seed untreated; (3) infected seed treated with 1-120 formaldehyde for two minutes at 50° C.; (4) infected seed untreated. Clean seed means merely tubers that proved to be free from sclerotia on careful examination after washing. It is not impossible that small sclerotia were sometimes overlooked, but every effort was made to select only tubers free from sclerotia. The same treatment that was used for the formaldehyde checks, two minutes at 50° C., was used for the furfural treatments. Furfural solutions of 1-200, 1-100 and 1-50 and 1-33 with a one and five hour cover for each concentration were used in 1924. Covering consisted in placing burlap sacks, which had been wet with the solution used in treating potatoes, around the treated potatoes for the required time. A furfuramide dust treatment was also used in the first year's tests. The treatments in the 1925 tests differed from those of the preceding year in that another check treatment was included, infected seed treated with 1-1000 mercuric chloride for two minutes at 50° C., and in that the 1-200 furfural and the furfuramide treatments were omitted.

Field plot technique as outlined by Melhus, Gilman and Kendrick (5) was followed in these tests. Four replications of each treatment were used for each variety tested. Each replication consisted of a single row 200 feet long and in which were planted 200 seed pieces. Data were taken on total yield and percent of tubers infected with black scurf. Each row was harvested separately by hand. The tubers were then placed on a screen and washed and carefully sorted into two lots; *Rhizoctonia* infected, and apparently clean.

The data of the 1924 potato seed treatments are given in Table XI. Student (9) method was used for determining significance of results. As there appeared to be little difference in varietal response to the different treatments, the three varieties were merged and 12 replications used in computing the results. Odds of more than 30:1 were regarded as significant. The only treatments significantly better than the infected formaldehyde check were the two for which clean seed was used. The only

TABLE X. TOXIC ACTION OF SEED DISINFECTANTS ON SEED VIABILITY IN TERMS OF PERCENT OF GERMINATION.

Treatment	Treatment			Tilletia tritici		Ustilago levis and U. avenae		Ustilago hordei Barley
	Oz. per bu.	Percent	Time cover hrs.	Spring wheat	Winter wheat	Oats		
1. Copper carbonate	2.00			95.0	69.0		86.0	93.5
2. "	3.00							
3. "	8.00			84.0				96.0
4. Furfuramide	1.00			88.0		85.5	91.0	
5. "	2.00							95.0
6. "	2.66			83.5	75.0	99.0		94.0
7. "	4.00			84.5		95.5		
8. "	8.00							94.5
9. Furfurine	1.00			84.5	63.0	91.5		
10. "	2.00			88.0	71.0	95.5		95.0
11. "	4.00			84.0	76.0	91.5		91.5
12. "	8.00			83.5		97.5		94.0
13. Furoin	8.00			84.5		88.0		
14. Furoic acid	2.00			72.5		88.5		69.0
15. Furfuracrylic acid	2.00						89.0	
16. Mercuric chloride	2.00			64.5			83.5	
17. "	5.33			14.5				
18. Mercuric chloride and copper carbonate (2:1)	3.00						81.0	
19. Mercuric chloride and Sil o'oil (2:1)	3.00						88.5	
20. Mercuric chloride and Furfuramide (2:1)	3.00			89.0			93.5	
21. Mercuric chloride and Furfuramide (2:1)	6.00						88.5	
22. Mercuric chloride and Furfuramide (2:1)	8.00			71.5				
23. Mercuric chloride and Furfuramide (2:1)	9.00							
24. No treatment	1.00			65.5	63.0	97.0	77.0	97.5
25. Copper stearate				94.5	71.0		90.5	

TABLE X—(Continued).

26. Copper stearate	2.00					89.5	77.0			93.0	
27. Mercuric furoate	2.00					93.0	87.5			89.5	
28. Ferric furoate	2.00					91.5	74.0			91.0	
29. Calcium furoate	2.00					91.0	87.5			87.0	
30. Nickel furoate	2.00					91.5	86.5			86.5	
31. Cupric furoate	2.00										
32. Mercuric furoate and calcium carbonate (1:4)	4.00					94.5	78.0			85.5	
33. Ferric furoate and calcium carbonate (1:4)	4.00					96.0	80.5			90.0	
34. Calcium furoate and calcium carbonate (1:4)	4.00					91.0	80.0			85.0	
35. Nickel furoate and calcium carbonate (1:4)	4.00					88.5	78.0			90.0	
36. Cupric furoate and calcium carbonate (1:4)	4.00					91.5	80.0				
37. Calcium carbonate	4.00					91.5	76.0			89.5	
38. No treatment					0						
39. Water sprinkle		2.0			0	92.5					
40. Furfural sprinkle		1.0			0	88.5		85.5			
41. " "		0.75			0	90.5		92.5			79.0
42. " "		0.50			0	91.0		94.0			91.5
43. " "		0.33			0	93.5		98.0			94.5
44. " "					3	90.5		96.0			94.5
45. Water sprinkle		2.00			3	92.0				90.5	89.0
46. Furfural sprinkle		1.00			3	81.5		73.0		76.5	
47. " "		0.75			3	85.0		94.0		83.5	
48. " "		0.50			3	89.0		91.0		80.0	
49. " "		0.33			3	92.0		93.0		91.5	
50. " "		0.03			3	87.0		95.0		94.5	
51. Formaldehyde		2.00			12	85.0		90.0		92.0	
52. Water sprinkle		1.00			12	94.0				93.5	
53. Furfural sprinkle		0.75			12	32.5		28.0		60.0	
54. " "		0.50			12	82.0		86.0		88.5	
55. " "		0.33			12	86.5		84.0		88.5	
56. " "					12	88.0		87.5		90.5	
57. " "					12	94.0		85.5		92.0	
58. No treatment		0.50			2	93.5		94.5		92.5	
59. Furfural soak							82.5	82.5		89.5	

TABLE X—(Continued).

Treatment	Treatment			Tilletia tritici				Ustilago levis and U. avenae		Ustilago hordei Barley
	Oz. per bu.	Percent	Time cover hrs.	Spring wheat		Winter wheat	Oats			
				1924	1925					
60. " "		0.10	2	63.5		80.5	94.0	1924	1925	1924
61. " "		0.05	2	67.0		81.0	95.5			88.0
62. Formaldehyde soak		0.50	2	30.5			79.5			87.0
63. " "		0.10	2	66.5		82.0	93.0			90.5
64. Formaldehyde dip		0.05	2	63.0		86.0			96.5	88.0
65. Water dip			6							87.5
66. Furfural dip		0.50	6	43.0		75.0			59.0	92.0
67. " "		0.10	6	64.5		84.5			88.5	94.5
68. " "		0.05	6	58.0		87.5			97.0	95.0
69. Formaldehyde dip		0.50	6	20.5					53.5	95.5
70. " "		0.10	6	53.5		83.5			86.5	84.5
71. " "		0.05	6	55.0		74.0			94.0	95.5
72. Formaldehyde spray, 1 qt. 50 bu.		50.0	5		90.5				94.0	98.0
73. Formaldehyde spray, 1 qt. to 50 bu.		95.0	5		90.0				94.5	

TABLE XI. EFFECT OF THE 1924 POTATO SEED TREATMENTS ON PREVALENCE OF RHIZOCTONIA AND THE SIGNIFICANCE OF THE VARIOUS TREATMENTS.

Condition of Seed	Fungicide	Dilution	Temperature degree	Time		Ave. Pct. infected in 12 replications	Mean variation from infected formaldehyde treated check	Standard duration	Factor $\frac{Z}{Z_{check}}$	Odds in favor of infected formaldehyde treated check
				Dipped minutes	Covered hours					
Infected	None	1-120	50	2	3	61.4	22.8	23.02	0.99	269:1
"	Formaldehyde	1-33.3	50	2	1	37.8				
"	Furfural	1-33.3	50	2	1	37.9	0.1	20.7	0.005	1:1
"	"	1-50	50	2	5	35.5	-2.3	22.3	0.10	1:1.7
"	"	1-50	50	2	1	44.4	6.6	22.4	0.27	4.1:1
"	"	1-50	50	2	5	35.2	-2.6	22.4	0.11	1.8:1
"	"	1-100	50	2	1	39.1	1.3	23.5	0.06	1:1
"	"	1-100	50	2	5	44.2	6.4	21.9	0.29	4.5:1
"	"	1-200	50	2	1	48.8	11.0	27.0	0.41	8.3:1
"	"	1-200	50	2	5	52.0	14.2	23.2	0.61	28.1:1
"	Furfuramide dust					59.6	21.8	16.0	1.36	2380:1
Apparently clean	None	1-120	50	2	3	25.4	12.4	17.0	0.73	1:56
Apparently clean	Formaldehyde					21.9	15.9	18.0	0.88	1:138



TABLE XII. EFFECT OF THE 1925 POTATO SEED TREATMENTS ON PREVALENCE OF RHIZOCTONIA AND THE SIGNIFICANCE OF THE VARIOUS TREATMENTS.

Condition of seed	Fungicide	Dilution	Temperature degree cent.	Time		Ave. Pct. infected in 4 replications	Mean variation from infected formaldehyde treated check	Standard deviation	Factor Z	Odds in favor of infected formaldehyde treated check
				Dipped minutes	Covered hours					
Infected	None	1-120	50	2	3	49.0	37.4	24.5	1.53	24.9:1
"	Formaldehyde	1-1000	50	2	5	11.6	17.5	7.95	2.20	61.9:1
"	Mercuric chloride	1-33.3	50	2	1	24.3	12.7	4.69	2.71	108.9:1
"	Furfural	1-33.3	50	2	5	26.9	15.3	6.28	2.44	80.9:1
"	"	1-50	50	2	1	31.1	19.5	5.0	3.9	302:0
"	"	1-50	50	2	5	28.9	17.3	8.33	2.09	54.3:1
"	"	1-100	50	2	1	43.3	31.7	6.91	4.58	54.3:1
"	"	1-100	50	2	5	36.2	24.6	1.29	19.0	Infinite
Clean	None	1-120	50	2	3	17.4	5.8	12.28	0.47	3.9:1
Clean	Formaldehyde	1-120	50	2	3	7.2	-4.4	6.67	0.66	1:4.9

The significance is computed in the "Students Method" pairing each treatment with infected formaldehyde 1-120 for two minutes at 50° to 52° C., followed by covering for three hours.

treatments significantly inferior to the infected formaldehyde check were the infected untreated and infected treated with furfuramide dust. The 1-200 furfural treatments, one and five hour cover, were markedly inferior to the formaldehyde check. The other furfural treatments controlled potato Rhizoetonia practically as well as did formaldehyde. The most effective treatment, where infected seed was used, was the 1-50 furfural, two minutes at 50° to 52° C. with a five hour cover which had 35.2 percent Rhizoetonia, 2.7 percent less than the formaldehyde treatment.

The results of the field trials in 1925 for the effect of seed treatment on the prevalence of Rhizoetonia of potatoes are given in Table XII. The results differ greatly from those of the preceding year in that the formaldehyde treatments were comparatively much more effective. The formaldehyde treatment of infected seed reduced the amount of Rhizoetonia 76 percent over the infected not treated, whereas in 1924 it caused a reduction of 38 percent. In 1925, 5.8 percent less Rhizoetonia developed from the formaldehyde treatment of infected seed than from the clean untreated check and only 4.4 percent more than from the clean seed treated with formaldehyde. In 1924 both of these treatments were significantly superior to the infected formaldehyde check treatment. The reduction in the amount of Rhizoetonia produced by the 1-33 and 1-50 furfural treatments was approximately the same for the two years. In 1924 these treatments caused reductions of 28 to 43 percent and in 1925 of 37 to 50 percent over the infected no treatment. Apparently the greater control in the 1925 treatments was due to a difference in the amount of soil infection. The land used in 1924 had been in potatoes for three successive years, while that used in 1925 had grown no crop of potatoes for at least ten years.

A statistical examination of the 1925 results using the Student (9) method of computing probable error, shows that the hot formaldehyde treatment is significantly better than any of the furfural or the hot mercuric chloride treatments. The small odds in favor of the formaldehyde treatment in comparison with the untreated infected check are probably due to the limited number of replications (four) and to the great variation in the percent of Rhizoetonia in the individual rows (22.5 to 75.4 percent of the infected no treatment check rows.) The differences between the infected formaldehyde treatment and the clean untreated and clean formaldehyde treatments were not significant. These trials coming through two seasons, although somewhat variable, indicate clearly that furfural and formaldehyde in equal concentrations are not equally effective for potato seed disinfection. Raeder et al (8) have also suggested that furfural under Idaho conditions is less effective than formaldehyde in equal concentrations. It required in their trials a 1-60 furfural solution for ten minutes at 50° C. to compare favorably with 1-120 formaldehyde for five minutes at 55° C. Higher concentrations of furfural as 1-33 or 1-50, should they prove as effective as formaldehyde, nevertheless introduce the item of cost, which at present is in favor of formaldehyde.

## SUMMARY

1. Toxicity studies were made using the following organisms: *Puccinia coronata holci*, *Ustilago hordei*, and *Sphacelotheca sorghi*, and dilute solutions of six aldehydes as follows: formaldehyde, butylaldehyde, benzaldehyde, furfuraldehyde, propylaldehyde and acetaldehyde. The aldehydes differed in their toxic action on *Puccinia coronata holci*, *Ustilago hordei* and *Sphacelotheca sorghi*. Benzaldehyde, formaldehyde and butylaldehyde appeared to be most toxic to the organisms studied, followed by furfuraldehyde, propylaldehyde and acetaldehyde.
2. Apparently the furan nucleus was a minor factor in the toxicity of furfural. Metallic furoate salts were markedly toxic and their toxicity was in accord with the toxicity of the metal carried. Furfuralcohol did not display the toxicity exhibited by the aldehyde, suggesting that the furyl radical is not the toxic portion of the aldehyde or alcohol.
3. Raising the temperature of the solution accelerated the toxicity of furfural to the sclerotia of *Rhizoctonia solani*. Laboratory tests indicated that covering increased the effectiveness of the furfural treatments.
4. The toxic effect of furfural and formaldehyde on germinating wheat seed is different. Injury with furfural vapor in the presence of water was greatly increased, while with formaldehyde vapor the reverse was true.
5. Seeds treated with formaldehyde solutions, dried and stored for various lengths of time before planting showed cumulative injury, while those treated with furfural did not.
6. Soaking the seed with water or furfural solutions ranging from 0.05 to 0.5 percent or sprinkling with water or furfural solutions ranging from 0.33 to 2.0 percent greatly increased the amount of bunt in wheat. Similar treatments on oats and barley had only slight effects on the percent of smut. Soaking or sprinkling with formaldehyde solutions either reduced or eliminated these smuts.
7. Dust derivatives of furfural, while in most cases reducing the percent of smut, did not give evidence of possessing sufficient toxicity to control these diseases.
8. Mercuric chloride dust or a mixture of two parts mercuric chloride dust to one part copper carbonate, Sil O'Cil, or furfuramide reduced oat smut considerably, but did not entirely eliminate it. Two ounces of mercuric chloride dust gave as good control as 5.66 ounces. Two ounces of mercuric chloride plus one ounce of copper carbonate was no better than two ounces of mercuric chloride alone. Two ounces of mercuric chloride plus one ounce of Sil O'Cil, as a spreader, gave fully as good results as the copper carbonate mixture. The addition of furfuramide to mercuric chloride apparently reduces the efficiency of the latter.
9. Mercuric chloride dust proved injurious to wheat and oat germinations in all cases.

10. Dust derivatives of furfural and copper carbonate were beneficial to germination of wheat when seed of poor quality was used.
11. When used under similar conditions, formaldehyde was more injurious to seed germination than equal concentrations of furfural.
12. Treatment of seed potatoes infected with *Rhizoctonia* with solutions of 1-100, 1-50 and 1-33 furfural for two minutes at 50° C. controlled *Rhizoctonia* as well as the 1-120 hot formaldehyde treatment in 1924. The 1-200 fufural and the furfuramide dust treatments were unsatisfactory. In the 1925 field trials the 1-120 hot formaldehyde treatment controlled *Rhizoctonia* better than did any of the other treatments. The 1-33 and 1-50 furfural and the 1-1000 mercuric chloride treatments for two minutes at 50° C. although materially reducing the amount of infection were significantly inferior to the formaldehyde treatment.
13. The length of cover after treatment had little effect on the amount of *Rhizoctonia*, which developed in the field experiments in either 1924 and 1925.

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## A SUMMARY OF THE SOIL FUNGI

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The key to soil fungi presented here is the outgrowth of a study of the fungus flora of soils which has extended over a period of nearly five years. During the course of the work several hundred isolations were made from soils in Iowa and Louisiana, while a less systematic study was made of soils from other sources as opportunity arose. The key is intended to include all of the species of fungi which are reported in the literature as having been isolated from the soil, in addition to those which are reported for the first time by the authors. Certain exceptions are noted under the different genera.

It is realized of course, that in the future many additional fungi will be isolated from the soil which are not included here. It is also probable that some species have been isolated from the soil which have not been formally reported in the literature. Studies of the fungous flora of soils in many parts of the world, however, have shown that a considerable number of species are widely distributed in soils, and it is believed that the more common soil organisms are included in the key.

Wherever possible the key has been based on the existing keys to the various genera and species, making such changes as were necessary to include the soil forms and the new species described. In some cases it was necessary to key species which had not been examined in culture, which is rather difficult in instances where the published descriptions are meager. The key to the genera is adapted from that given in Engler and Prantl (18).

The term "soil" is not defined. It is quite probable that the meaning of the term as used in some of the European literature differs somewhat from the common idea of soil in America, but it would be impossible to differentiate between them for the purposes of this paper. However, species of fungi were excluded from the key which are reported in the literature as having been isolated from material that had not been incorporated into the soil proper and was still recognizable, such as decayed wood and leaf mold. Such organisms are listed as doubtful soil fungi.

A group of organisms which are probably soil inhabiting are many of the fleshy fungi, particularly members of the families Agaricaceae and Lycoperdaceae, which are found commonly in meadow soils and woodlands. The relationship between the mycelium of these fungi and their substratum is not sufficiently clear to definitely determine whether they should be classified as soil fungi or more properly as symbionts associated with the roots of various flowering plants in a mycorrhizal condition. It was therefore thought best to omit them from this list.

Nor has any attempt been made to include those plant pathogenic fungi which are reported in the literature as occurring in the soil, but which have

been isolated from the soil only by means of their attack on the particular host plant growing in soil infested by them. There is a considerable number of such organisms, particularly among the species of the genera *Fusarium* and *Pythium*.

Only such forms as have been directly isolated on an artificial cultural medium are included. It is recognized that in all work of this type the medium used exerted a selective action on the species reported; hence it should be noted that in the isolations made by the authors, Waksman and Fred's (54) synthetic acid agar was employed throughout.

This agar is recommended for the isolation of fungi from the soil because it eliminates bacterial colonies and favors the growth of fungi. For the cultural studies Czapek's solution agar and dextrose bean agar were used. As a rule morphological characters do not differ greatly on these media, although growth is usually more abundant on the bean agar. A few organisms failed to grow at all typically on Czapek's agar.

The soils studied in Iowa and Louisiana are described in previous publications (2), (3). The fungi reported from Utah were isolated from five representative types of cultivated soil from that state, furnished through the courtesy of Dr. Thomas L. Martin, Brigham Young University, Provo, Utah.

Two hundred and forty-two species of fungi in 61 genera have been described from the soil. The following 20 species new to science are described in this paper:

<i>Phoma humicola</i> n.sp.	<i>Glocladium atrum</i> n.sp.
<i>Coniothyrium terricola</i> n.sp.	<i>Sporotrichum pruinatum</i> n.sp.
<i>Monilia implicata</i> n.sp.	<i>Periconia lanata</i> n.sp.
<i>Monilia brunnea</i> n.sp.	<i>Acremoniella brevia</i> , n.sp.
<i>Penicillium crateriforme</i> n.sp.	<i>Dematium scabridum</i> n.sp.
<i>Penicillium restrictum</i> n.sp.	<i>Mesobotrys simplex</i> n.sp.
<i>Penicillium guttulosum</i> n.sp.	<i>Helminthosporium anomalum</i> n.sp.
<i>Penicillium vinaceum</i> n.sp.	<i>Spondylocladium australe</i> n.sp.
<i>Glocladium catenulatum</i> n.sp.	<i>Acrothecium robustum</i> n.sp.
<i>Glocladium fimbriatum</i> n.sp.	<i>Volutella piriformis</i> n.sp.

Cultures of these species have been deposited with the American Type Culture Collection, at the John McCormick Institute for Infectious Diseases at Chicago, Illinois.

Twenty-six of the species described have not previously been reported from the soil and 58 are reported from new localities.

The distribution of species among the genera listed is worthy of comment. Species of the genus *Penicillium* were most numerous, numbering 39; the genus *Mucor* was a close second with 32 species, *Fusarium* was third, being represented by 27 species, and *Aspergillus* was fourth, with 20 species. The other genera were represented by from one to six species each.

#### KEY TO THE CLASSES, ORDERS, FAMILIES, AND GENERA OF SOIL FUNGI

- a. Filaments one-celled, rarely septate; conidia usually in sporangia; sex-cells when present uniting to form resting-spores.

##### A. *Phycomycetes*.

- b. Conidia typically in globose to cylindric sporangia; zygosporous.

##### I. *Mucorales*.

- c. Sporangia always present, conidia sometimes present.
  - d. Columella present; zygospore naked or with a few appendages.
  - e. Wall of the sporangium homogeneous, not cuticularized, diffuent.
  - f. Sporangia similar.
- I. *Mucoraceae* p. 234.
- g. Sporangia pyriform.
1. *Absidia*.
- gg. Sporangia spherical.
  - h. Sporangiphores united into groups on a rhizoidiferous stolon. Sporangiphores at the nodes opposite the rhizoids. Spores usually longitudinally striate.
2. *Rhizopus*.
- hh. Sporangiphores emerging singly from the mycelium, not from rhizoidiferous stolons. Spores generally smooth, without longitudinal striae.
  - i. Zygospores, the result of the union of two equal gametes.
3. *Mucor*.
- ii. Zygospores, the result of the union of two unequal gametes.
4. *Zygorhynchus*.
- ff. Sporangia of two sorts, primary and secondary.
- II. *Thamnidaceae* p. 253.
- g. A single genus.
5. *Thamnidium*.
- ee. Wall cuticularized and persistent above, thin and diffuent below.
- III. *Pilobolaceae* p. 253.
- f. A single genus.
6. *Pilaira*.
- dd. Columella absent; zygospore enveloped in a dense covering.
- IV. *Mortierellaceae* p. 254.
- e. A single genus.
7. *Mortierella*.
- cc. Sporangia rarely present, conidia always present.
  - d. Conidia solitary; zygospore arising directly from the gametes.
- V. *Chaetocladiaceae* p. 256.
- e. A single genus.
8. *Cunninghamella*.
- bb. Conidia single or in chains on conidiophores; sporangia when present give rise to zoospores; oosporous.
- II. *Peronosporales*.
- c. Vegetative mycelium very narrow, uniform, much branched; sporangiphores not distinct from mycelium; zoosporangia or conidia single on the tips of the branchlets, producing zoospores or a germinating tube; oospores globose with a well developed outer wall.
  - d. Conidia elongate, globose or lemon-shaped; contents escaping in-

to a globose vesicle in which the zoospores arise, zoospores 2-ciliate.

VI. *Pythiaceae* p. 257.

e. A single genus.

9. *Pythium*.

aa. Filaments septate; conidia borne on conidiophores, sex-cells rarely in evidence.

b. Spores in a definite number in a sac: as ascospores.

B. *Ascomycetes*.

c. Asci in a perithecium; perithecium dark-colored, either without a stroma, partly seated in a loose mass of mycelium, or sessile above an imperfect stroma.

III. *Sphaeriales*.

d. Walls of perithecia thin and membranous, asci soon disappearing.

e. Perithecia always superficial, with mouth surrounded by long, branched, hooked or spiral setae.

VII. *Chaetomiaceae* p. 261.

f. A single genus.

10. *Chaetomium*.

ee. Perithecia usually sunken, with only short hairs about the mouth.

VIII. *Sordariaceae* p. 263.

f. Stromata absent, spores simple.

11. *Pleurage*.

ff. Stromata absent, spores 4- many celled.

12. *Sporormia*.

cc. Asci in a perithecium, perithecium bright colored, free or in a stroma.

IV. *Hypocreales*.

d. Stromata wanting; or when present with perithecia entirely superficial.

IX. *Nectriaceae* p. 265.

e. A single genus.

13. *Neonectria*.

bb. Spores as conidia on conidiophores, of various form, not in asci.

C. *Fungi Imperfecti*.

c. Conidia present.

d. Conidia in globoid, cup-shaped or hysteroioid pycnidia.

V. *Sphaeropsidales*.

e. Pycnidia typically membranous to carbonous, dark, brown or black.

f. Pycnidia more or less globose, rarely cylindric.

X. *Sphaeroidaceae* p. 265.

g. Conidia 1-celled, hyaline, globose, ovoid or oblong, often curved.

h. Pycnidia separate, smooth, not rostrate, conidia less than 15 $\mu$  in length.

14. *Phoma*.

- gg. Conidia 1-celled, dark, globose, ovoid, or oblong.  
 h. Pycnidia separate without subicle, smooth, conidia borne on short conidiophores.

15. *Coniothyrium*.

- hh. Pycnidia separate without subicle, hairy or setose.

16. *Chaetomella*.

dd. Conidia not in pycnidia.

- e. Conidiophores not on a matrix, typically well developed, but sometimes short or even lacking.

VI. *Moniliales*.

- f. Conidiophores in more or less loose cottony masses.

- g. Conidiophores and conidia clear or bright colored.

XI. *Moniliaceae* p. 267.

- h. Conidia hyaline or bright colored, 1-celled, globose, ovoid or short cylindric.

Hyalosporae.

- i. Conidiophores very short or obsolete, or little different from the conidia.

Micronemeae.

- j. Conidia in chains.

Oosporeae.

- k. Conidiophores short, simple or nearly so.

17. *Oospora*.

- kk. Conidiophores longer, distinctly branched.

18. *Monilia*.

- ii. Conidiophores elongate and distinct from the conidia.

Macronemeae.

- j. Conidiophores unbranched or only slightly branched, often swollen at the tip. Conidia often in heads.

Cephalosporiaceae.

- k. Conidia not in chains, but single.

- l. Conidia without sterigmata.

- m. Conidia not inclosed in mucus.

- n. Conidiophores simple.

19. *Cephalosporium*.

- nn. Conidiophores branched.

20. *Trichoderma*.

- kk. Conidia produced in chains.

Aspergilleae.

- l. Conidiophores distinctly swollen at the tip, foot cells prominent.

21. *Aspergillus*.

- ll. Conidiophores not or only slightly swollen at the tip, foot cells not differentiated; tips usually verticillately branched.

- m. Conidiophores with tips equally verticillate; spores cask-shaped.

22. *Amblyosporium*.



- mm. Conidiophores with tips unequally verticillate.
- n. Conidia not enclosed in mucus.
  - o. Conidiophores not branched, except at tip.
    - 23. *Penicillium*.
  - oo. Conidiophores freely branched.
    - 24. *Spicaria*.
- nn. Conidia enclosed in mucus, chains of conidia not always distinguishable.
  - 25. *Gliocladium*.
- jj. Conidiophores more or less richly branched.
  - k. Conidia not formed on special intercalary cells but usually terminal.
    - l. Branching of the conidiophores very manifold.
      - Botrytideae.
    - m. Conidia smooth.
      - n. Conidiophores decumbent.
        - 26. *Sporotrichum*.
      - nn. Conidiophores erect.
        - o. Conidio borne singly.
          - 27. *Monosporium*.
        - oo. Conidia in heads.
          - 28. *Botrytis*.
      - m. Conidia warted.
        - 29. *Sepedonium*.
    - ll. Branching of the conidiophores only in whorls.
      - Verticillieae.
    - m. Conidia solitary or loosely grouped, not in chains.
      - n. Conidia-bearing branches very short, flask-shaped sterigmata.
        - 30. *Pachybasium*.
      - nn. Conidia-bearing branches longer, cylindrical.
        - o. Spores single, easily scattered.
          - 31. *Verticillium*.
        - oo. Spores enclosed in mucus to form heads.
          - 32. *Acrostalagmus*.
    - kk. Conidia formed on differentiated intercalary cells of the conidiophore.
      - Gonatobotrytideae.
      - l. Conidiophores of sterile and fertile cells: sterile cells bone-shaped, swollen at the ends: fertile cells without sterigmata.
        - 33. *Nematogonium*.
  - hh. Conidia not 1-celled, more or less elongate.

- i. Conidia 2-celled, ovoid or short fusoid  
Hyalodidymae.
- j. Conidia smooth.
  - k. Conidia single and terminal on sharply differentiated mycelium. 34. *Trichothecium*.
- jj. Conidia warty. 35. *Mycogone*.
- ii. Conidia more than 2-celled, oblong, fusoid or elongate.  
Hyalophragmiae.
- j. Conidiophores distinct from the conidia.  
Macronemeae.
- k. Conidia ovate cylindric or elongate, often catenate. 36. *Ramularia*.
- gg. Hyphae and conidia both typically dark or one or the other always dark.

XII. *Dematiaceae* p. 313.

- h. Conidia dark, or sometimes hyaline but the hyphae then dark, 1-celled, globose to oblong.  
Amerosporae.
- i. Vegetative hyphae long; conidiophores present and differentiated from the mycelium.  
Macronemeae.
- j. Conidia dark colored.
  - k. Conidia not in chains.
    - l. Conidia in terminal heads.  
Periconiaeae.
  - m. Conidiophores simple.
    - n. Apex with sterigmata.
      - o. Conidia not enveloped in slime. 37. *Stachybotrys*.
      - oo. Conidia enveloped in slime. 38. *Gliobotrys*.
    - nn. Apex short branched or simple, not swollen. 39. *Periconia*.
  - m. Conidiophores branched.
    - n. Conidiophores forked below the apex; conidia oblong. 40. *Synsporium*.
    - nn. Conidia inserted irregularly on jar-like basidia; conidia globose. 41. *Basisporium*.
- ll. Conidia single and terminal on unbranched conidiophores.  
Monotosporeae.

- m. Sterile hyphae lacking, conidia black.  
42. *Acremoniella*.
- k. Conidia in chains.
  - l. Conidiophores unbranched, conidial chains lateral.  
43. *Dematium*.
  - ll. Conidiophores dendroidly branched.  
44. *Hormodendrum*.
- jj. Conidia hyaline or almost hyaline; conidiophores dark colored.
  - k. Conidia single, not in chains and heads.  
Chloridieae.
    - l. Conidiophores branched only in the center; conidia ovoid.  
45. *Mesobotrys*.
- hh. Conidia dark, more than one-celled.
  - i. Conidia 2-celled, ovoid or oblong.
    - j. Conidiophores very short or little differentiated from the conidia.  
Micronemeae.  
Bisporeae.
    - k. Conidia borne singly, conidiophores not swollen.  
46. *Dicoccum*.
  - jj. Conidiophores distinct from the mycelium, usually erect.  
Macronemeae.
  - k. Conidia, smooth walled, not in heads.  
Cladosporieae.
    - l. Conidia usually not in chains, terminal and lateral, conidiophores much branched.  
47. *Cladosporium*.
    - ll. Conidia borne terminally on thread-like sterigmata.  
48. *Scolecobasidium*.
- kk. Conidia more than 2-celled; ovoid, cylindric or vermicular.
  - l. Conidia with cross walls only; not muriform.  
Phragmosporeae.
    - m. Conidia formed singly, either terminal or lateral.  
Helminthosporieae.
    - n. Conidia smooth, straight, elongate; conidiophores rigid.  
49. *Helminthosporium*.
  - mm. Conidia formed in terminal heads of lateral whorls.  
Acrothecieae.

- n. Conidia formed in lateral whorls.  
50. *Spondylocadium*.
  - nn. Conidia formed in terminal heads.  
51. *Acrothecium*.
- ll. Conidia muriformly divided; globose to oblong.  
Dictyosporae.
- m. Conidiophores distinct from the mycelium.  
Macronemeae.
  - n. Conidia of one sort, occurring singly.
  - o. Conidia, cruciately divided, warted.  
52. *Tetracoccusporium*.
  - oo. Conidia muriform, usually smooth.
  - p. Conidiophores decumbent.  
53. *Stemphylium*.
  - pp. Conidiophores erect or ascending, conidia ovoid to oblong.  
54. *Macrosporium*.
  - nn. Conidia in chains, caudate; conidiophores erect.  
55. *Alternaria*.
- ff. Hyphae compactly united to form a globose to cylindric body which is often stalked.
- g. Hyphal body cylindric to capitate, stalked, i.e., a synnema.  
XIII. *Stilbaceae* p. 326.
- h. Hyphae and conidia dark colored.  
Phaeostilbeae.
  - i. Conidia 1-celled globose, or ovoid.
  - j. Conidia in chains, borne on a brush-like coremium.  
56. *Stysanus*.
  - jj. Conidia not in chains, borne on single side-branches.  
57. *Tilachlidium*.
- gg. Hyphal body more or less globose, sessile, i.e. a sporodochium.  
XIV. *Tuberculariaceae* p. 327.
- h. Hyphae and conidia hyaline or pale colored.  
Mucedineae.
  - i. Conidia 1-celled; globose or ovoid.
  - j. Sporodochium without hairs or bristles; conidiophores not on a plectenchymatic hypothecium.  
58. *Hymenula*.
  - jj. Sporodochium covered with hairs or bristles; conidiophores forming a covered hymenium, hymenium not stromate.  
59. *Volutella*.
- ii. Conidia more than 2-celled.  
Phragmosporeae.

j. Conidia fusiform curved; sporodochium waxy or cottony.

60. *Fusarium*.

hh. Hyphae and conidia dark colored.

Dematiaceae.

i. Conidia 1-celled.

j. Sporodochium surrounded with hyaline hairs or bristles.

61. *Myrothecium*.

cc. Conidia lacking.

VII. *Mycelia sterilia* p. 339.

d. Sclerotia present.

e. Sclerotia tubercle-like.

62. *Rhizoctonia*.

## A. PHYCOMYCETES

### I. Mucorales

The classification of the Mucorales is largely that used by Lendner (27) except that the genus *Zygorhynchus* is recognized. Lendner's keys have been used as a basis for the construction of a key to the soil forms.

### I. MUCORACEAE

#### 1. *Absidia* van Tieghem 1876 (27)

Mycelium formed as in the genus *Rhizopus* by frequently branched stolons, more or less incurved into archs and producing at the point of contact with the substratum more or less richly branched rhizoids. Sporangiphores straight, rarely single, more often in groups of 2-5, occurring at the curve of the stolon (internodal) and not at the point of origin of the rhizoid (nodes). At times there occur erect stolons or branches which bear lateral sporangiferous branches which may be confused with the primary sporangiphores. Sporangia apparently equal, piriform, erect, furnished nished with an infundibuliform apophysis. Membrane of the sporangium not cuticularized nor inerusted; diffluent, leaving a short basal collarette. Columella hemispheric, conic or mammi-form, more rarely spinescent or terminated by a single long prolongation. It effaces itself in the apophysis. It is cuticularized and its color is more pronounced than that of the sporangiphore. A cross-wall is placed at a definite distance below the sporangium. Spores, small, 5-6 $\mu$ , round or oval (not angular) with a smooth wall, rarely echinulate, colorless or bluish-black. Zygospores formed on the stolons. They are surrounded by circinate filaments, cutinized, which are borne in a whorl from one or both of the



Fig. 1. *Absidia*. a-habit sketch; b-sporangiphore; c-spores; d-zygospore. (After Lendner).



suspensors. Gametes straight. On germination the zygospores produce either mycelial filaments or sporangiophores. Apparently closely related to the genus *Rhizopus*, differing from that genus by the fact that the sporangiophores occur on the internodes, by the piriform sporangia, by the continuance of the columella into the apophyses and the suspensors having circinate filaments.

#### KEY TO THE SPECIES OF THE GENUS *ABSIDIA*

- a. Spores elongate cylindric; appendages on only one of the suspensors.
  - 1. *A. spinosa*.
- aa. Spores oval or globose; appendages, where known, on both suspensors.
  - b. Columella furnished with a single terminal prolongation.
    - c. Prolongation of columella short, pointed; columella globose, mam-miform.
      - 2. *A. glauca*.
    - cc. Prolongation longer, rounded at tip, columella turbinate.
      - d. Spores globose 2.5-3.5 $\mu$  in diameter.
        - 3. *A. orchidis*.
    - dd. Spores globose, larger 4-7 $\mu$ .
      - 4. *A. coerulea*.
  - bb. Columella smooth or rarely faintly spinescent.
    - c. Spores generally globose, rarely oval, 3-4 $\mu$  in diam. Columella generally spinescent.
      - 5. *A. lichtheimii*.
    - cc. Spores irregular; columella smooth.
      - 6. *A. subpoculata*.

#### \*1. *Absidia spinosa* Lendner (27)

Syn. *A. cylindrospora* Hagem

Turf very close, the filaments interlacing into a greyish cottony mat, about 2½ cm. above the substratum. Stolons little curved, arched, carrying the sporangia in groups of 2 or 3. Sporangia pear-shaped, bluish, 34 $\mu$  long, from the apophysis to the end of the sporangium, by 28 $\mu$  wide. Columellae 20 $\mu$  wide, swollen, ending in a blunt or rounded spine, reaching 1/3 the length of the columellae. Septa present, 25 $\mu$  below the apophyses separating the sporangia from the sporangiophores. Spores hyaline, oval or short rods, sometimes very slightly constricted in the middle, 2 $\mu$  in diameter by 4-5 $\mu$  long. Zygospores spherical or doliform, verrucose, formed by the fusion of two unequal gametes on a forked hyphae. The larger suspensor furnished with circinate appendages.

From soil: Norway (22), Switzerland (27)

United States: Hawaii (53), Idaho (36)

#### \*2. *Absidia glauca* Hagem (27)

Culture on wort gelatin grey-green when young (10 days) then becoming clear yellow brown. The stolons present the same kind of branching

\* Species marked (\*) not studied by the writers.

as *Absidia orchidis*. The fertile branches are either isolated, or in groups of two, three or four. Sporangia piriform, measuring 40-50 $\mu$  in diameter by 44-60 $\mu$  in length. A septum dividing the pedicel from the sporangium is formed a distance equal to half the length of the entire apophysis. Wall encrusted with granules, is diffuent and leaves a very straight collarete. Columella rounded, mammiform, furnished with a very short button; it measures at least 30 $\mu$  in diameter by 38 $\mu$  in length. Spores, round, 3-3.5 $\mu$ , colorless. Heterothallic.

From soil: England (16), Norway (22), Switzerland (27).

United States: Idaho (36)

\*3. *Absidia orchidis* (Vuillemin) Hagem (27)

Primary axes 0.6 to 10 mm. long, straight or more or less changed into irregular stolons, at times raised and indefinitely elongate; at times curved toward the substratum to which their end is attached by a tuft of rhizoids; at times erect and ended by a sporangium. These stolons are branched sympodially and bear sterile or fertile branches, the latter occurring singly or in groups of 2 or 3. Sporangioophores simple or bearing at some distance from the tips an oblique branch, shorter than the tip of the principal pedicel. This latter branch also ends in a like sporangium. Septa divide the pedicels at a distance from the infundibuliform apophyses a little greater than the height of the apophyses themselves.

Sporangia ovoid, 40 $\mu$  in height by 32 $\mu$  in diameter (in the case of the large sporangia). Wall encrusted with fine granules, imperfectly diffuent, leaving a straight, rigid, collarete. Columellae conic, rounded, longer than broad, surmounted usually by a knob; attenuated or constricted at the base and remaining upright when after dehiscence the columella becomes free. Spores slightly brownish, perfectly spherical, varying from 2.5 $\mu$  to 3.5 $\mu$  in diam. Heterothallic.

From soil: England (16), Norway (22)

United States: New Jersey (52) (53)

\*4. *Absidia coerulea* Bainier (27)

Filaments of the thallus bluish-violet, continuous, unequally branched, at times knotted. Sporangioophores single, borne directly on the thallus, attaining 25 mm. in length, ended by an infundibuliform apophyses. Septa 12-24 $\mu$  from the tip. Sporangia uniform, globular, 36-42 $\mu$ , changing from pale violet to grey, then to brown. Membrane of the sporangium smooth, diffuent, leaving a collarete. Columellae hemispheric or obconic, often ending in a papilla. Spores numerous, small, smooth, pale violet, globose, 4-7 $\mu$ . Zygospores 60 $\mu$ , brown, globose, rugose-verrucose. Suspensors straight, enlarging into barrel-shape, furnished with 10-20 circinate appendages, long and thin (7 $\mu$  in diameter), arranged in a single verticil. Azygospores similar. Chlamydospores smooth intercalary.

From soil: Holland (32)

\*5. *Absidia lichtheimii* (Lucet and Constantin) Lendner (27)

Sporangioophores prostrate, branched in corymbs, forming a white felt, woolly. They terminate the corymbiform branching by carrying the sporangia on longer or shorter pedicels. A little below the terminal corymb frequently there occur groups of branches carrying smaller sporangia.

Sporangia erect, hyaline, pear-shaped, with an infundibuliform apophysis, becoming attenuate gradually to the sporangiophore. Average diam.  $45\text{--}60\mu$ , the greatest  $70\mu$ , the least  $10\text{--}20\mu$ . Wall of sporangium colorless, transparent, smooth, diffuent, leaving a basal collarette. Columellae large, hemispheric or globular,  $10\text{--}20\mu$ , smooth (or furnished with short spines) smoky grey or brown. The apophysis and pedicel also similarly colored. Spores spheric, subspheric or more rarely oval, colorless, small, usually  $2\mu$  in diam. by  $3\mu$  long (sometimes larger,  $4\text{--}6.5\mu$ ). Zygosporoes not known.

From soil: Switzerland (27)

United States: Maine (52) (53), New Jersey (52) (53)

## 5. *Absidia subpoculata* Paine (33)

Colonies white floccose, aerial hyphae growing to a height of 1.5 to 2 cm. floccose. Stolons branched with sporangiophores occurring in groups of 1-5. Sporangiophores branched,  $100\text{--}300$  long by  $4\mu$  in diam. with a septum  $10\text{--}12\mu$  below the tip. Sporangia globose  $22\text{--}24\mu$  in diam. with a smooth diffuent wall, leaving a slight collarette. Columellae oval, slightly constricted at the apophyses; without apophyses  $4\text{--}7 \times 8\text{--}9\mu$ , with apophyses  $10\text{--}20 \times 7.5\text{--}15\mu$ . Apophyses rounded below into a distinct pouch. Spores oval to spherical to allantoid,  $2\text{--}2.5 \times 3\text{--}4\mu$ . Chlamydospores quite numerous, spherical,  $4\text{--}5\mu$  in diameter.

From soil: United States: Iowa (33), Louisiana

## 2. *Rhizopus* Ehrenberg 1820 (27)

Mycelium of two kinds, one submerged in the substratum and the other aerial, constituting the arching filaments or stolons. These stolons present from place to place the nodes on which occur the rhizoids, which are implanted in the substratum. At these points the sporangiophores arise. They may be single but usually occur in groups of 2, 3 or more. The summit of the sporangiophore is enlarged into an apophysis, of the kind that has the columella inserted above the point where the spherical bend attaches into the filament. The sporangia, white at first, become bluish-black at maturity. They are all the same size, spherical or almost spherical, flattened at the base. Wall not cuticularized, uniformly incrustated and entirely diffuent, without leaving a basal collarette. Columella broadly subjaacent, hemispherical, forming after dehiscence, by collapse, an organ of the shape of the pileus of a basidiomycete. Spores round or oval, angular, colorless or colored bluish or brown, with a cuticularized wall, smooth or striate, rarely spinulose. Zygosporoes naked, formed in the substratum and on the stolons. Suspensors straight, very large and swollen, without appendages.

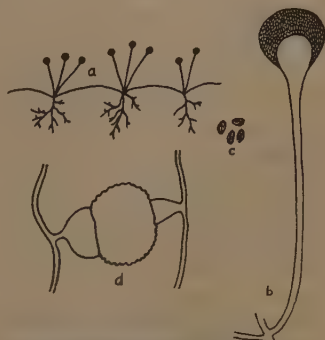


Fig. 2. *Rhizopus*. a-habit sketch; b-sporangiophore; c-spores; d-zygospore.

## KEY TO THE SPECIES OF THE GENUS RHIZOPUS

a. Rhizoids well developed.

1. *R. nigricans*

aa. Rhizoids little developed or lacking.

b. Rhizoids rare, pale, short; sporangiophores without swellings.

2. *R. arrhizus*

bb. Rhizoids rare; sporangiophores and stolons branched and swollen in places.

3. *R. nodosus*1. *Rhizopus nigricans* Ehrenberg (27)

Stolons creeping, recurving to the substrate in the form of arachnoid hyphae, which are strongly raised and distant from the substrate and implanted at each node by means of rhizoids. The internodes often attain a length of 1-3 cm. and the hyphae are more or less branched. Sporangiophores rarely single, united in groups of 3-5 or more, 0.5-4 mm. in height by 24-42 $\mu$  in diam. Apophyses broad, cuneiform. Sporangia hemispheric 100-350 $\mu$ . Columellae broad, hemispheric, depressed, 70 $\mu$  in diam. by 90 $\mu$  in height (max. 250 x 320 $\mu$ ). Spores unequal, irregular round or oval, angular, striate, 9-12 $\mu$  long by 7.5-8 $\mu$  in diam., of a grey blue. Zygospores round, or oval, 160-220 $\mu$  in diam. Exospore brown black, verrucose. Suspensors swollen, usually unequal. Azygospores present. No chlamydospores.

From soil: Germany (5), Japan (45), Norway (22)

United States: California (53), Hawaii (53), Idaho (36), Iowa (1) (3) (53), Louisiana (2) (53), New Jersey (28) (52) (53), New York (26) North Dakota (53), Oregon (52) (53), Rhode Island (38), Texas (56)

\*2. *Rhizopus arrhizus* Fischer (27)

Differs from *R. nigricans* by its less exuberance. The felt is clearer and it does not extend so far into the substrate. Stolons are little developed and do not form nodes regularly. Rhizoids pale, develop at the nodes and carry sporangia, or are sometimes formed indeterminately. Sporangiophores often prostrate, rarely single, forming umbels or corymbs on their stolons. They measure 0.5-2 mm. in length. All the branches end in sporangia, of greater or less size. Sporangia spherical 120-250 $\mu$  in diam. Columellae spherical, flattened on the apophyses, 40-75 $\mu$  high x 60-100 $\mu$  in width, membrane brown, smooth. Spores round or oval, or presenting obtuse angles, greyish brown; walls striated longitudinally, 4.8-7 $\mu$  x 4.8-5.6 $\mu$ .

From soil: England (16), Hungary (30)

3. *Rhizopus nodosus* Namyslowski (27)

The mycelium is cottony, white when young, then tinted ochre yellow. In the midst of the mycelium and on the stolons, branches ending in sporangia occur. These branches 1-2 mm. in height by 12-28 $\mu$  in diam. have thick, smooth walls, colorless at first, then becoming pale ochre or brown. They are simple or branched, the branches ending in sporangia. The branches may be swollen at any point. When these swellings are terminal they give rise to a group of 3-5 sporangiophores, each terminating in a sporangium. Sporangiophores 1-2 mm. high, the sporangia are globose

100-200 $\mu$  in diam. The spores 6-9 $\mu$  long by 4-6 $\mu$  in diam. striated longitudinally. They may give rise to chlamydospores 16-32 $\mu$  in diameter. Zygo-spores 120-140 $\mu$  occur. They are round, oval or without definite shape. The suspensors are equal or different in size and shape.

From soil: Norway (22), Switzerland (27)

United States: New Jersey (52) (53), Oregon (53)

### 3. *Mucor* Micheli 1729 (27)

Mycelium widespread in and on the substratum, but without roots or especial membered stolons; richly branched, with branches always thinner until at last hair-fine; straight or knotted, at first one-celled, in age with irregular cross-walls, with colorless, infrequently orange-red content; smooth, colorless membrane. Sporangiohores springing singly from the mycelium but usually forming a thick turf, erect, either unbranched with terminal sporangia or branched with like sporangia on all the branch ends; branching in part monopodial, clustered or irregularly paniced or umbelliferous; in part cymose and more or less sympodial, curved, with sporangia also at the tip of the sympodium, never forked. Sporangia erect, at times in sympodial sporangiohores, a few weakly bent, usually all alike, only of different size; many spored, spherical, opening on the sporangio-phore, only a few in sympodial forms, abscissing while still closed; of various colors. Sporangial wall not cuticularized, incrustated more or less strongly with needles of calcium oxalate, dissolving quickly in water, leaving a collarette, or breaking and then at times persistent. Columellae always present, of various shape, colorless or colored. Spores spherical or ellipsoid with thin smooth membrane, colorless or colored. Zygosporos on the mycelium, not on special branches, naked; suspensors without outgrowths; gametes straight. Mycelial conidia (stylospores) unknown. Gemmae (chlamydospores) terminal and intercalary, variously formed, colorless, smooth, not in all species.

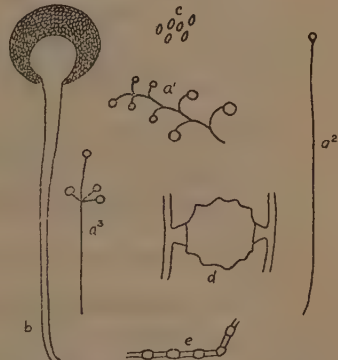


Fig. 3. *Mucor*. a1-Racemo-mucor; a2-Mono-mucor; a3-Cymo-mucor; b-sporangio-phore; c-spores; d-zygospore; e-chlamy-dospores.

#### KEY TO THE SPECIES OF THE GENUS MUCOR

- a. Sporangiohores not branched.
  - A. Mono-Mucor.
- aa. Sporangiohores branched.
  - b. Branches rare or more numerous and then indefinite, in clusters or corymbs.
    - B. Racemo-Mucor.
  - bb. Branches definite, in sympodia.
    - C. Cymo-Mucor.



A. *Mono-Mucor*

- a. Sporangioophores collapsing early to form a woody felt, reddish in color.
  - 1. *M. rufescens*.
- aa. Sporangioophores remaining erect to form a turf.
  - b. Sporangioophores less than 300 $\mu$  high.
    - c. Sporangioophores 5-6 $\mu$  in diam., sporangia small about 20 $\mu$  in diam.
      - 2. *M. ramannianus*.
    - cc. Sporangioophores 2-2.5 $\mu$  in diam., sporangia larger, usually up to 30 $\mu$  in diam.
      - 3. *M. mirus*.
  - bb. Sporangioophores more than 300 $\mu$  high.
    - c. Sporangioophores not more than 2 cm. high.
      - 4. *M. adventitius*.
    - cc. Sporangioophores more than 2 cm. high.
      - d. Sporangioophores more than 2-3 cm. high. Sporangia 80 $\mu$  in diam.
        - e. Membrane of sporangium non-diffuent.
          - 5. *M. microsporus*.
        - ee. Membrane of sporangium diffuent.
          - 6. *M. hiemalis*.
      - dd. Sporangioophores more than 3 cm. high. Sporangia 250-350 $\mu$  in diam.
        - 7. *M. mucedo*.

B. *Racemo-Mucor*

- a. Secondary branches whorled, these latter carrying whorls of branches in their turn.
  - 8. *M. glomerula*.
- aa. Branching plainly in clusters or corymbs.
  - b. Spores very unequal (a mixture of numerous small spores beside others more than twice as large).
    - c. Sporangioophores usually 3-4 mm. long; sporangia up to 70 $\mu$  in diam.
      - 9. *M. sylvaticus*.
    - cc. Sporangioophores usually 1 cm. long; sporangia up to 54 $\mu$  in diam.
      - 10. *M. lausannensis*.
  - bb. Spores apparently equal.
    - c. Wall of sporangium not diffuent but breaking into pieces.
      - d. Sporangia very variable, columellae usually globose.
        - 11. *M. racemosus*.
      - dd. Sporangia of uniform size, columellae elongate.
        - 12. *M. christianiensis*.
    - cc. Wall of sporangium diffuent.
      - d. Sporangioophores very high, 6-8 cm.
        - 13. *M. flavus*.
      - dd. Sporangioophores not over 3 cm. high.
        - e. Sporangioophores thin, 5-8 $\mu$  in diam.
          - f. Spores large, 11-13 $\mu$  in diam.
            - 14. *M. dispersus*.

- ff. Spores small, 3-6 $\mu$  in diam.
  - 15. *M. echinulatus*.
- ee. Sporangioophores thicker, 8-25 $\mu$  in diam.
  - f. Spores elongate, planoconvex, 9-10 x 3-4 $\mu$ .
    - 16. *M. genevensis*.
  - ff. Spores elliptic or oval, 4-6 x 3-4 $\mu$ .
    - g. Turf yellow to buff.
      - 17. *M. varians*.
    - gg. Turf smoky gray.
      - 18. *M. abundans*.

### C. *Cymo-Mucor*

- a. Sporangioophores not erect, forming a cobwebby network.
  - 19. *M. botryoides*.
- aa. Sporangioophores erect, some or all of them.
  - b. Sporangioophores of two kinds, one erect and carrying normal sporangia, the other prostrate, usually richly branched sympodially.
    - 20. *M. saturninus*.
  - bb. Sporangioophores of a single kind.
    - c. Sporangioophores circinate.
      - d. Sporangioophores not more than 1 cm. long, spores oval, 6 $\mu$  in maximum length.
        - e. Wall of sporangium brown, spores usually subsessile, 3-4 $\mu$  x 5-6 $\mu$  (Compare *M. sylvaticus*).
          - 21. *M. circinelloides*.
        - ee. Wall of sporangium blue-black, sporangia supported by long pedicels.
          - f. Sporangia 60-80 $\mu$  in diam., spores 4-6 x 4 $\mu$ .
            - 22. *M. griseocyanus*.
          - ff. Sporangia 50-60 $\mu$  in diam., spores 5-7 x 3.5-5 $\mu$ .
            - 23. *M. corticolus*.
        - dd. Sporangioophores from 1 to 3 cm. long; spores round, 10 $\mu$  or more in diam.
          - 24. *M. lamprosporus*.
      - cc. Sporangioophores straight, not circinate.
        - d. Spores round, apparently alike.
          - e. Growth on wort gelatin, poor, turf on bread short, 2-3 mm.
            - 25. *M. jansseni*.
          - ee. Growth on wort gelatin, good; turf 1-3 cm.
            - f. Columellae spinescent.
              - g. Sporangioophores less than 2 mm. high; spores smooth.
                - 26. *M. spinescens*.
              - gg. Sporangioophores 1 cm. high, spores slightly echinulate.
                - 27. *M. plumbeus*.
            - ff. Columellae smooth.
              - g. Sporangioles present.
                - h. Sporangia 70-110 $\mu$  in diam., sporangioles not evanescent.
                  - 28. *M. sphaerosporus*.

hh. Sporangia not exceeding 80-90 $\mu$ ; sporangioles evanescent; circinate.

24. *M. lamprosporus*.

g. Sporangioles not present.

29. *M. dimorphosporus*.

d. Spores oval.

e. Spores elongate, echinulate.

30. *M. ambiguus*.

ee. Spores sub-spheric with smooth membrane.

f. Sporangia 50-350 $\mu$  in diam., columellae globose.

31. *M. geophilus*.

ff. Sporangia 90-174 $\mu$  in diam., columellae ovoid.

32. *M. strictus*.

\*1. *Mucor rufescens* Fischer (27)

Sporangiophores not branched, flaccid collapsing to form a cottony felt with a reddish color, 2.5 cm. long by 15 to 25 $\mu$  in diameter. They are often irregularly divided by septa which separate the collapsed portion from the turgid filaments; wall colorless, contents being furnished with orange-red colored drops; sporangia large, 120 to 150 $\mu$  in diameter, pale yellow hyaline, walls of sporangium slowly diffuent, slightly incrustated, colorless, hyaline. Columella free, globose or elliptical, spherical or sub-spherical, 45 to 65 $\mu$  in diameter, with a smooth, colorless wall. Contents dense, intensively colored golden yellow, which is seen through the sporangium wall and gives it its colored appearance. Spores plano-convex, with obtuse tips, and twice as long as broad, 4 $\mu$  broad up to 10 $\mu$  long, but may be 8 $\mu$  in breadth to 21 $\mu$  in length, colorless, smooth. Zygospores and chlamydospores unknown.

From soil: England (15) (16)

2. *Mucor ramannianus* A. Moeller (27)

Turf, short, velvety, of a carmine red brown. The edge of colony white, becoming grey with age. Sporangiohores unbranched, less than 200 $\mu$  long by 5-6 $\mu$  in diam. Sporangia very small, usually 20 $\mu$  in diam. (max. 40 $\mu$ ), spherical, flesh-rose to copper-red. Wall, unequally diffuent, smooth. Spores globose, rarely oval, 2-3 $\mu$  in diam., colorless (the coloration of the sporangia is probably due to some interstitial substance). Columella spherical, of variable size, 8-10 $\mu$  in diam. Chlamydospores numerous, 10-12 $\mu$  in diam., globose or ovoid. Giant-cells are often present. Mycelium and sporangiophores are rather frequently septate.

From soil: England (15), Norway (22)

United States: Iowa (33), Maine (53), Michigan (35)

3. *Mucor mirus* Paine (33)

Colonies flat, except they are usually papillate in the center, zonate, quite spreading, velvety, ashy gray scarcely any elevation. Sporangiohores unbranched, arising in the margin of the colony in large numbers but not numerous inside the margins, rather delicate and slender, 125 to 300 $\mu$  long and 2 to 2.5 $\mu$  thick. Sporangia smoky, spherical, 10 to 30 $\mu$  in diam. and appear slightly rough; the sporangial wall is diffuent, leaving no collar. Columella spherical, 5 to 12 $\mu$  in diam. Spores quite small, 2.5 to 3.5 $\mu$  in diam., often oval and slightly pointed at the ends. Chlamydospores are

exceedingly numerous, and appear as large globular swellings in the subterranean hyphae 20 to 35 $\mu$  in diam. and appear to contain 2 to 8 angular spore-like bodies varying from 4 to 12 $\mu$  across, rather angular in shape.

From soil: United States: Iowa (33)

\*4. *Mucor adventitius* Oudemans (27)

Sporangiophores simple, continuous, hyaline, forming a turf 20 mm. high. Sporangia globose, 80 to 95 $\mu$  in diam. at first hyaline, later light gray, finely echinulate, with a diffluent membrane. Columellae at first globose, later elliptic or companulate, hyaline with colorless content, 40-48 $\mu$  by 48-64 $\mu$  and furnished with a basal collarette. Spores elliptic or nearly oblong 8-8.5 x 4.5-5 $\mu$  smooth, hyaline greyish when in mass. Zygosporos and chlamydospores unknown.

From soil: Holland (32), Japan (45)

\*5. *Mucor microsporus* Namyslowski (27)

Colonies whitish, with age becoming yellowish especially when grown on pears; mycelium cottony; sporangiophores unbranched, up to 2 cm. high, 12-20 $\mu$  thick, below the columella strongly attenuated; sporangia brownish, 30-80 $\mu$  in diameter, mostly 60 $\mu$ ; membrane of young sporangia not diffluent; with age, however, it dissolves; columellae spherical, somewhat higher than broad, beneath weakly attenuated, always with flat base and short collar; smooth, 20-70 $\mu$  broad, often filled with brick-red contents; spores regularly ellipsoidal, hyaline, smooth, 2-3 $\mu$  long, 1.5 $\mu$  broad, (max. 4 $\mu$  long), in mass when young ashen-gray, with age bluish.

From soil: Austria (30)

United States: Maine (53), New Jersey (52) (53)

6. *Mucor hiemalis* Wehmer (27)

Sporangiophores usually unbranched, erect, then prostrate by wilting. Turf about 1 cm. high (0.5-2 cm.) close and fine, cottony white, rarely greyish yellow. Sporangia spherical grey or brownish yellow, visible to the naked eye, 52 $\mu$  in diam. Wall diffluent in young condition, leaving a collarette. Columella free, spherical or oval, colorless, 28-48 $\mu$  (spherical) or 25 x 21 $\mu$  to 36 x 29 $\mu$ . Spores usually unequal, the majority elongate, ellipsoid or kidney shaped, 7 x 3.2 $\mu$  (limits 3-8.4 x 2-5.6 $\mu$ ) smooth, hyaline, with thin membrane. Mycelium comes to resemble that of *M. rouxianus* by the accumulation of oil drops.

From soil: Austria (30), Norway (22), Switzerland (27)

United States: Iowa (33), Maine (53), New Jersey (52) (53), New York (26)

\*7. *Mucor mucedo* (Linne) Brefeld (27)

Sporangiophores erect, forming a very raised turf up to 15 cm. in height, silvery grey, shining, not branched, 2-15 cm. high by 30-40 $\mu$  in diam., without cross walls; wall colorless, smooth; content colorless, tardily yellow. (Very rarely branched with very small sporangia). Sporangia large, 100-200 $\mu$  in diam.; at first yellow, then deep grey or brownish black. Membrane of sporangium very diffluent, leaving a collarette; it is encrusted with needle-shaped crystals of calcium oxalate. Columellae free, cylindric

or campanulate or spherical, 70-140 $\mu$  long by 50-80 $\mu$  wide, with colorless wall and red orange content. Spores elliptic or sub-cylindric, twice as long as broad, of very various sizes in the same sporangium, 6-12 $\mu$  long by 3-6 $\mu$  wide (limits 16.8 $\mu$  long) with a smooth hyaline wall, content tardily yellow or colorless. Zygosporangia spherical 90-250 $\mu$  in diam. Exospore black; thickly, and very strikingly verrucose; hard and fragile. Endospore colorless with less striking warts, enclosed in the former. On germination the zygosporangia give rise to sporangia on an unbranched sporangiophore. Chlamydospores not known.

From soil: Austria (30), Germany (5), Norway (22)

United States: New York (26)

8. *Mucor glomerula* (Bainier) Lendner (27)

Sporangiophores erect, very branched. Each erect branch terminated by a very large sporangium, below which occur a whorl of 3-8 secondary filaments, each terminated by a sporangium. These 3 to 8 filaments give rise in their turn to a whorl of 3-5 sporangiferous filaments. The aerial mycelial filaments usually end in branches carrying nearly sessile sporangioles. Sporangia spherical, hyaline, becoming sienna color when old. Wall roughened by crystals of calcium oxalate, diffuent leaving a collarette. Columellae variable in shape, hemispheric, cylindro-conic, ovoid, sometimes restricted, inserted at the rather suddenly expanded end of the sporangiophore. Spores round and smooth. Aerial chlamydospores round, with thick wall, yellow and spiny. Content oleaginous. Mycelial chlamydospores seemingly submerged but very numerous. Zygosporangia unknown.

From soil: England (16)

United States: Alaska (53), Iowa (1) (3), New Jersey (52) (53)

\*9. *Mucor sylvaticus* Hagem (27)

Turf white or grey formed of thin slightly dense filaments, extending over the surface. Sporangiophores rarely straight, but mostly irregularly incurved, branching near the tip with one or two lateral branches. They reach 1 cm. in height by 10 $\mu$  in width. Sporangia small, globose not more than 70 $\mu$  (average 44 $\mu$ ). Wall diffuent leaving a basal collarette. Spores of very variable size, oval or sub-globose 4 x 2 $\mu$ -5 x 3 $\mu$  (max. 8 x 6 $\mu$ ). Columellae globose or oval, 30 x 22 $\mu$ -20 x 25 $\mu$  in diam. At the point of contact with the substrate the chlamydospores are numerous. They are ovoid 16-24 $\mu$  in diam. The erect filaments frequently have large swellings which become isolated and form round cells measuring 40-60 $\mu$  in diam., rarely longer.

From soil: Norway (22), Switzerland (27)

United States: Louisiana (53), New Jersey (52) (53)

\*10. *Mucor lausannensis* Lendner (27)

Sporangiophores erect, little branched, bearing laterally one or two groups of branches. These sporangiophores form a fine compact turf, yellowish,  $\frac{1}{2}$  to 1 centimeter high (10-14 $\mu$  in diam.). Sporangia 40-54 $\mu$  in diam., often flattened at the base. The wall is not diffuent but fragile as in *M. racemosus*, leaving an irregular basal collarette. Columellae oval or spherical, 30-40 $\mu$  in diam. by 50 $\mu$  long. Spores oval, of very different



sizes, the smallest  $4 \times 2\mu$ , the largest  $12\mu$  long by  $6\mu$  wide. The average size is  $8 \times 6\mu$ . They are hyaline, then pale, slowly turning yellowish in mass. Chlamydospores, rather rare, may be formed on either the mycelium or the sporangiophore. They measure on the average  $16 \times 14\mu$ , are smooth and granular in content. Zygosporos not known.

From soil: England (16)

11. *Mucor racemosus* Fresenius (27)

Sporangiophores erect, close, forming a yellow brown turf of very variable height, 5-40 mm. high by  $8-20\mu$  wide, branched irregularly in groups. All the branches terminated by sporangia which are very unequal. Sporangia small, globose, unequal  $20-70\mu$  in diam., erect, or at times incurved, pale yellow, then wax-yellow or yellow brown, hyaline. Wall of sporangium not diffuent, but fragile, persistent, encrusted, leaving a collarette. Columellae free, globose, ovoid or broadly cuneiform, campanulate  $17-60\mu$  long by  $7-30\mu$  at the base and  $9-42\mu$  in greatest diam. Spores rarely globose, more often elliptic,  $5-8\mu$  by  $6-10\mu$ , smooth, yellow in mass. Zygosporos globose;  $70-85\mu$ , brown, with conic warts yellow or red-brown, suspensors straighter than the zygosporos and not swollen. Azygosporos, chlamydospores always very numerous, the latter formed either on the mycelium or on the sporangiophores and even on the columellae; they are colorless, or yellow, with a smooth membrane, of diverse shapes,  $20\mu$  in diam. or  $11-20\mu$  in diam. by  $20-30\mu$  in length. Budding cells formed in liquid sugars. The mycelium breaks up into oidia.

From soil: Canada (53), England (16), Germany (5), Japan (45), Norway (22), Switzerland (27)

United States: Alaska (53), California (53), Colorado (53), Hawaii (53), Iowa, (3), Maine (53), New Jersey (28) (52) (53), New York (26), Oregon (52) (53), Texas (53) (56).

\*12. *Mucor christianiensis* Hagem (24)

Colonies at first forming thick, superficial, grey mycelial mat, then a sparse formation of sporangiophores. Sporangiophores usually scattered, seldom forming a loose turf, 1.5-2.5 cm. high, exceptionally thin,  $6-10\mu$  thick, and quickly collapsing, unbranched when young; monopodially branched later with small, short circinate branches, with numerous chlamydospores on the sporangiophore, at first cylindric, later barrel-shaped or usually globose, a rather regular distance apart,  $50-200\mu$ , quickly freed when mature. Sporangia small, usually  $40-60\mu$  in diam., bright yellow when ripe with fragile wall, leaving only a small fragment at the base of the columellae. Columellae oval or usually elongate, always longer than wide,  $30-45\mu$  high and  $25-30\mu$  wide. Spores broad, oval, or some almost spherical,  $6-9\mu \times 5-7\mu$ . Zygosporos unknown.

From soil: Norway (24)

\*13. *Mucor flavus* Bainier (27)

Sporangiophores 8 cm. high by  $22-24\mu$  wide, little or not branched, at first colorless, then ochre yellow. Sporangia globose, grey, bluish, then white with a blue tint,  $140-160\mu$  in diam. Wall diffuent, encrusted, leaving a collarette. Spores oval, very variable in size  $9.4-12\mu$  by  $4.2\mu$ , sometimes cylindric or reniform, enclosed in an interstitial mucilaginous sub-

stance, very fluid, giving the sporangium a translucent blue appearance. Columellae at first globose, then slightly oval, (Lendner reports them rather pear-shaped, with a flattened base,  $110\mu \times 90\mu$ ). In liquid sugar media, budding cells as in yeast occur. Zygosporoes formed as in *M. racemosus*. Wall formed of numerous plates brown and deeper at the center of the zygosporoe, develops echinulations like those of *M. mucedo* rather tardily,  $150\mu$  in diam.

From soil: Norway (22), Switzerland (27)

United States: Maine (53), New Jersey (52) (53)

\*14. *Mucor dispersus* Hagem (24)

Sporangiophores small or slightly thickened turf of various heights. The larger (primary) 2-3 cm. high are widely scattered, very delicate, 5-7 $\mu$  in diam., waving here and there, usually soon collapsing, branched in monopodial clusters, with short bent, circinate, often secondarily branched branchlets; the primary as well as secondary branches terminate in sporangia. The smaller (secondary) sporangiophores, 1-2 mm. high, usually circinate, with small sporangia. Sporangia on primary sporangiophores small, about  $50\mu$ , with diffluent wall and smaller, usually truncate or broadly globose, seldom oval 17-19 $\mu$  high by 18-21 $\mu$  broad columellae, with small collarettes. Sporangia of the side branches as well as those of the secondary sporangiophores of various size, 15-45 $\mu$ , with spiny not diffluent wall and translucent spore mass, frequently very small with only 2-4 spores but also larger 30-45 $\mu$  with numerous spores. Spores of tolerably different size, 11-13 $\mu$ , usually round or slightly elongate, some even rounded-angular. Zygosporoes unknown.

From soil: Norway (24)

15. *Mucor echinulatus* Paine (33)

Colonies somewhat spreading soon developing a grayish-white powdery appearance, elevation of the aerial hyphae never exceeding 2 mm. in height; creeping, quite fragile turf. Sporangiphores monopodially branched, 150-400 $\mu$  in length by 5.5 to 8 $\mu$  thick, much vacuolated. Sporangia spherical 40-50 $\mu$  in diam., minutely echinulate, the spines being about 2 $\mu$  long, wall diffluent, leaving no collar. Columellae spherical, quite variable in size, 15-25 $\mu$  in diam. Spores ellipsoidal 3 to 3.5 $\mu$  by 4.5 to 5.5 $\mu$ , hyaline. Chlamydospores quite numerous in old cultures, cylindrical in form, quite variable in length.

From soil: United States: Iowa (33)

\*16. *Mucor genevensis* Lendner (27)

Turf close white 2 cm. high. Sporangiphores 2 cm. long by 10-15 $\mu$  wide, little branched in groups, carrying 1 or 2 lateral sporangia. Sporangia globose, 66 $\mu$  in average diam., but exceptionally reaching 80 $\mu$  in diam. Wall diffluent, almost colorless, rather yellow, leaving collarette. Columellae oval or round, free, colorless, 30-36 $\mu$  in diam. or 24 x 36 $\mu$ . Spores elongate, planoconvex, 9-10 $\mu$  long by 3-4 $\mu$  wide. Chlamydospores frequent, rather than oidiospores which are borne on lateral branches. Zygosporoes frequent on adjacent branches but not on forks of the same branch, 100 $\mu$  in diam. Epispore very thick, with conic warts.

From soil: Norway (22), Switzerland (27)

\*17. *Mucor varians* Povah (35)

Turf 1-3.5 cm. tall on bread, ivory yellow to olive buff; sporangiophores 8-20 $\mu$  in diam., either little or profusely branched, much coiled, twisted or intertwined, forming a dense tough cottony turf with proliferations of hyphae and columellae often present; sporangia globose or sub-globose, smooth, 60-80 $\mu$  in diam., at first yellow or pale orange, then very dark gray tinged with green, at maturity; wall diffuent leaving a basal collarette. Columellae free or slightly adnate, very variable in shape sub-globose, hemispherical, flattened hemispherical, oval, cylindrical, elliptical, pyriform, panduriform, cylindroconical, sub-conical and conical, large columellae hemispherical to conical, small columellae cylindrical to pyriform and panduriform 25-50 x 20-45 $\mu$ , membrane tinged gray, with or without orange contents. Spores not uniform oval to sub-elliptical 4-6 x 3-4 $\mu$ . Zygospores not found.

From soil: United States: Michigan (35)

\*18. *Mucor abundans* Povah (35)

Forming on bread a dense erect smoke-gray turf tinged drab 1.5-3.5 cm. tall. Sporangiophores 8-23 $\mu$  in diam., at first simple, later with one to three lateral branches which are in turn branched once or twice, with branches always terminating in a sporangium, and with a septum above point of insertion of branch; sporangia globose or sub-globose, smooth or encrusted with very delicate crystals, 56-78 $\mu$  in diam., at first yellowish, becoming dark gray with a greenish tinge at maturity. Wall diffuent, leaving a collarette; columellae sub-globose to pyriform, free or slightly adnate 31-40 x 25-35 $\mu$ , hyaline or tinged gray; spores variable, globose to short elliptical 3-5 $\mu$  in diam. or 4-5.5 x 3-4.5 $\mu$ . Chlamydospores and yellowish globules in submerged mycelium. Zygospores not found. Related to *M. hiemalis* (Sense of Hagem) from which it differs in the shape of the columellae and in the shape and size of the spores.

From soil: United States: Michigan (35)

\*19. *Mucor botryoides* Lendner (26)

Sporangiophores non-erect, incurved, forming a cobwebby network, attaining 1.5 cm. in height, not varying far from 16 to 20 $\mu$  in diam., ending in a larger sporangium and producing at a short distance below the sporangium more or less closely clustered branches that are terminated by sporangia; secondary branches are sometimes dichotomous or sometimes in sympodia; sporangia globose, clear gray, with membrane of the large as well as the small sporangia diffuent in water; average size of the terminal sporangia 80 $\mu$  in diam.; the lateral sporangia are variable and some are very small; columellae variable in form and size; hyaline, globose, campanulate or panduriform, 60 by 44 $\mu$ , or 55 by 38 $\mu$ , or 30 by 20 $\mu$ , and much smaller, usually without basal collar; spores hyaline, globose, average 8 $\mu$ , max. 10 $\mu$ , not rarely encountered as small as 5-6 $\mu$ ; appearing polyhedral because of their surface presenting a very slight roughness.

From soil: Switzerland (27)

United States: Alaska (53), Idaho (36), New Jersey (52) (53), New York (26), Rhode Island (38).

\*20. *Mucor saturninus* Hagem (24)

Colonies always more or less dark colored, usually lead-gray or lead-black, but sometimes even blue-black. Sporangophores of various heights, some low, others high. The lower from 1-2 mm. high usually richly branched monopodially or sympodially and forming a lead-black or blue-black turf from the large sporangia. The higher 2-3 cm. high, are more or less scattered, 20-25 $\mu$  thick and at first erect, later bent, branched monopodially with long branches and of a characteristic bright lead-grey color. Sporangia of the lesser branches at first bright waxy-yellow, then blue-gray, and finally almost black at maturity, of very different sizes, usually 45-180 $\mu$  in diam., with a spiny non-diffuent wall. Sporangia of the higher branches with a diffuent wall, leaving only a collarete. Columellae oval, seldom cylindrical, in the sporangia on the higher branches, frequently collapsed on the base, 60-100 $\mu$  high by 50-90 $\mu$  broad; the secondary (smaller sporangia) somewhat smaller, 35-70 $\mu$  high by 25-50 $\mu$  broad. Spores regularly broad ellipsoid (4.5-5)6-8(-10) x (3.5)4-6 (7) $\mu$  as well as a few small globose forms 4-4.5 $\mu$  in diam. Zygosporoes not known.

From soil: Norway (24)

United States: New Jersey (53)

21. *Mucor circinelloides* van Tieghem (27)

Sporangiophores erect, forming a very short turf, close and deep brown, about 1 cm. tall. They are more or less branched in sympodia with branches alternating right and left, short and more or less curved, always terminated by a sporangium. The length of the secondary branches is very variable; they are sometimes so short that the sporangium is seemingly sessile. Sporangia globose, 50-80 $\mu$  in diam., grey brown when walled; erect, or slightly incurved. The larger have a diffuent membrane; in the case of the smaller (the upper) the wall is persistent and the sporangia are evanescent. Wall of sporangium encrusted and when diffuent it leaves a basal collarete; but when not encrusted, persistent, firm and smooth. Columellae free, hemispheric or spheric or oval, colorless, smooth. Spores globose or elliptic, 3 $\mu$  in diam. by 4-5 $\mu$  long (Lendner, 4 x 5-6 $\mu$ ), smooth, colorless when single, but pale grey in mass. Zygosporoes globose, exospore red-brown, covered with very prominent spiny warts, longitudinally striate. Chlamydospores smooth, colorless, deep on the length of the filament. Gemmae as in yeasts and *M. racemosus*.

From soil: England (15) (16), Japan (45), Switzerland (27)

United States: Colorado (53), Idaho (36), Louisiana (53), Maine (53), New Jersey (52), (53), New York (26), North Dakota (53), Oregon (53)

\*22. *Mucor griseo-cyanus* Hagem (27)

Turf, deep grey blue, about 1 cm. high. Sporangophores branched, the longer in groups or in sympodia; the shorter always in sympodia. The lateral branches of the latter are rather circinate. Sporangia globose, 60-80 $\mu$  in diam., with a non-diffuent wall, encrusted with very small crystals of calcium oxalate. They are strongly colored grey-blue. Columellae round or ovoid, flattened at the base and conerescent with the membrane of the sporangium, 30-40 $\mu$  long by 24-36 $\mu$  wide, colored a clear fuliginous brown. Spores oval brown in masse, 5-6 $\mu$  long by 4 $\mu$  wide. Chlamydospores formed



on the sporangiophores and the filaments of the mycelium, oval or round, 12-14 $\mu$  in diam. Zygospores unknown.

From soil: Norway (24), Switzerland (27)

\*23. *Mucor corticolus* Hagem (24)

Colonies grey or slightly blue-grey; sporangiophores erect, up to 2 cm. high, sympodially branched with small, long branches, terminating in a sporangium. Lateral branches, long (2-3 times as long as *M. silvaticus*) and usually 600-1500 $\mu$  long by 10-15 $\mu$  thick, often more or less curved and terminating with sporangia. Sporangia globose, 50-60 $\mu$  in diam., with diffuent wall. Columellae egg-shaped or slightly oval, almost always 3-6 $\mu$  longer than broad, 27-33 $\mu$  wide by 30-36 $\mu$  long, without or with colorless content and usually with an indistinct collarette. Spores oval or elliptic (larger than *M. silvaticus*) 5-7 by 3.5-5 $\mu$ . Zygospores not known.

From soil: Norway (24)

United States: Michigan (35)

\*24. *Mucor lamprosporus* Lendner (27)

Turf about 3 cm. above the surface of the substrate, a dense felt, pale gray. Sporangiophores rather irregularly branched in groups or in sympodia, 3 cm. high, branches alternate, recurved, bearing minute sporangia. Sporangia terminal, globose, 60 $\mu$  in diameter (90 $\mu$  max.). Wall diffuent. Columellae spheric, 20 $\mu$  in diam., or ovoid, 24 $\mu$  wide by 28 $\mu$  long. Lateral sporangia, minute, 30-40 $\mu$  in diam., deciduous. Spores globose, colorless, hyaline 10 $\mu$  in diam. (7-12 $\mu$ , limits).

From soil: Switzerland (27)

\*25. *Mucor jansseni* Lendner (27)

Turf very short, velvety, becoming yellow to orange with age. Sporangiophores 2-6 mm. high, much branched in corymbs or in sympodia; ending in sporangia. Wall obliquely striate. Sporangia globose, deep bluish black, 50-70 $\mu$  in diam. Wall finely granular, not diffuent, but fragile. Columellae sometimes round, with a wide flattened base, subadjacent; sometimes elongate and conic, tinted deep blue or gray, 34 $\mu$  long by 30 $\mu$  wide; the smaller are proportionately longer, 20 $\mu$  wide by 26 $\mu$  long. Spores round, 5-6 $\mu$  usually, sometimes smaller, 3-4 $\mu$  in diam.

From soil: Switzerland (27)

United States: Idaho (36)

\*26. *Mucor spinescens* Lendner (27)

Turf very short, 1-2 mm. in height. Sporangiophores branched and short, maximum 1 mm. long by 10 $\mu$  wide, thinner near the sporangium. It is rarely straight, often slightly incurved. Sporangia globose variable in size (60-64 to 68 $\mu$  in diam.). Spores rather large, 7-8 $\mu$ , rarely smaller, 5-6 $\mu$ , slightly colored, clear yellow brown. Columellae at times ovoid, at times pear-shaped, or even elongate; often with a varying number of prolongations on their tips. Zygospores unknown. Differs from *M. jansseni* by its spinescent columellae and larger spores; from *M. plumbeus* by its much smaller sporangiophores.

From soil: United States: Idaho (36)



27. *Mucor plumbeus* Bonorden (27)

Turf close, regular, mouse grey, about 1 cm. deep. Sporangiophores erect, 1 cm. long, branched in groups or in sympodia. All the branches terminated by sporangia. Wall, smooth, colorless. Sporangia 100 to 300 $\mu$  in diameter, deep brown or black. Wall diffluent, encrusted, leaving a basal collarette. Columellae free, oval or pear-shaped, furnished at their summit with a variable number of spines (up to 12 or more) irregular, often swollen at the tip, 22-85 $\mu$  long by 8-65 $\mu$  wide; they are often colored grey or brown. Spores globose, equal, 5-8 $\mu$  (exceptionally 9-12 $\mu$ ) grey-blue with a dotted wall. Zygosporangia globose, yellow brown; exospore furnished with irregular warts in the shape of plates. Chlamydospores formed on the mycelium or the sporangiophores as in *M. racemosus*. Budding cells as in yeasts.

From soil: England (15)

United States: California (52), Idaho (36), New Jersey (52) (53), New York (26)

\*28. *Mucor sphaerosporus* Hagem (27)

Turf short, rather deep brown,  $\frac{1}{2}$  cm. high. Sporangiophores branched sympodially or in corymbs of 3-5 branches; about 1 cm. high by 15-18 $\mu$  wide, with wall colored a pale brownish-red. Sporangia globose, brownish-red, 70-110 $\mu$  in diam. Wall diffluent in the case of the large sporangia, fragile and persistent in the case of the small, stippled. Columellae ovoid or round, free at the base or quite campanulate with a flattened base (40-65 $\mu$  high by 30-55 $\mu$  broad, Hagem). Spores round, (very exceptionally, ovoid), very shining, tardily reddish, and rather equal, 10 $\mu$  (6-8 $\mu$ ). Sporangia numerous on the substrate but not evanescent. Chlamydospores and oidiospores numerous. Zygosporangia unknown.

From soil: England (15) (16), Norway (22)

United States: Idaho (36), New Jersey (52) (53)

\*29. *Mucor dimorphosporus* Lendner (27)

Turf grey, about 2 cm. high. Sporangiophores branched in sympodia, erect, 2 cm. long by 12 $\mu$  wide. Near the surface of the substrate, other wavy or circinate sporangiophores occur bearing smaller sporangia. Sporangia normally spheric, not more than 80 $\mu$  in diam., often rather wider than long, 60 by 58 $\mu$  in height on the average. Wall diffluent. Spores usually spheric, 8-10 $\mu$  in diam., often oval 6x8 or 8x10 $\mu$ , hyaline or slightly yellow, shining. Abnormal spores 30 $\mu$  long by 8-10 $\mu$  wide, very irregular, are found. Columellae rather larger toward the base, 20 x 24-40 x 50 $\mu$ , oval, encircled by a collarette. Chlamydospores on the sporangiophores. Zygosporangia not known.

From soil: Switzerland (27)

\*30. *Mucor ambiguus* Vuillemin (27)

Sporangiophores erect, forming a blackish turf, 1 mm. in height, branched in sympodia and bearing 4-5 sporangia, branches short, straight, or slightly incurved. Sporangia globose, 100 $\mu$  in diam., grey-black. Wall of sporangia more or less encrusted and more or less diffluent. The successive sporangia are more and more persistent, the wall finally dehiscing by fragmentation. Columellae free, globose, or campanulate. Spores elliptic 4.5 $\mu$  wide by 7 $\mu$  long, with a finely stippled wall. Zygosporangia unknown.

From soil: United States: Michigan (20)

31. *Mucor geophilus* Oudemans (27)

Mycelium snow white, very tardily grey, finely pale olive. Sporangio-phores simple or branched in cymes, carrying 2-3 branches. Sporangia globose, at first yellow, then olivaceous, leaving a collarette after the destruction of the membrane, dimensions 50-350 $\mu$  in diam. Wall with small blunt warts. Columellae globose, voluminous, pale grey. Spores pluriform, globose, round or elliptic, angular, 4.2-6.5 $\mu$  in diam., smooth, olive. Chlamydospores on the branches of the mycelium, round, 20 $\mu$  in diam., at times in a more or less extended series. Zygosporangia very like chlamydospores, about 30 $\mu$  in diameter.

From soil: Holland (32)

United States: Iowa (1) (3)

\*32. *Mucor strictus* Hagem (22)

Turf greyish white, about 1 cm. high,  $\frac{1}{2}$  below the surface of the substrate and dotted with sporangia; at first white, then pale brown; dark brown with age. Sporangio-phores simple or branched sympodially up to 1 cm. high by 16 $\mu$  wide, slightly incurved at the tip, restricted by the insertion of the sporangia. Wall of sporangio-phores striately netted. Sporangia globose, spheric, rather flattened on the side toward the columellae, 70 $\mu$  high by 88 $\mu$  broad, up to 170 $\mu$  in diam. Wall diffuent but not in all the sporangia. Columellae ovoid, rather flattened at the base, subjacent, 60 x 44 $\mu$  or 64 x 50 (max. 140 x 110) $\mu$ . Spores subspheric or oval, slightly unequal, 5 x 6 $\mu$  or 6 x 8 $\mu$ , rarely 10 $\mu$ . No chlamydospores. Zygosporangia unknown.

From soil: Norway (22)

4. *Zygorhynchus* Vuillemin 1903 (18)

Hyphae continuous, branched, unequal, often nodose, immersed, prostrate or forming a cottony aerial turf. Chlamydospores smooth, intercalary or terminal. Sporangio-phores solitary or in an irregular sympodial system, bearing typical sporangia or abortive sporangia and zygosporangia; not apophysate. Sporangia uniform; wall, diffuent, with the base conerescent with the columella. Upon its disappearance a collarette remains. Spores numerous, minute, smooth. Zygosporangia variable, warted. Gametes very unequal produced on unequally bifurcated hyphae, one straight and small, the other curved and thicker, at the end a reflexed pear-shape.

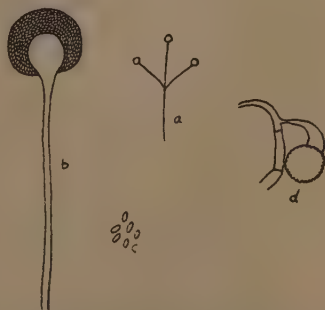


Fig. 4. *Zygorhynchus*. a-habit sketch; b-sporangiophore; c-spores; d-zygosporangium.

KEY TO THE SPECIES OF THE GENUS *ZYGORHYNCHUS*

- a. Spores globose; 2-3 $\mu$  in diam.
  - 1. *Z. heterogamus*.
- aa. Spores elongate.
  - b. Spores 4 x 2 $\mu$ ; zygosporos 40-50 $\mu$  in diam., with prominently verrucose epispore.
    - 2. *Z. vuilleminii*.
  - bb. Spores 5 x 3 $\mu$ ; zygosporos averaging 35 $\mu$  in diam., with less prominently verrucose epispore.
    - 3. *Z. moelleri*.

\*1. *Zygorhynchus heterogamus* Vuillemin (27)Syn. *Mucor heterogamus* (Vuill) Lendner

Sporangiophores erect, 2 mm. long by 12-15 $\mu$  wide, sometimes simple, and ending in a sporangium, more often branched, bearing 2 and at times 4 branches opposite each other or in whorls, all ending in sporangia. Sporangia equal, globose, 50-60 $\mu$  in diam., black. Wall of sporangium diffuent, exerusted, leaving a collarete. At the time of zygosporos formation the wall becomes persistent. Columellae spherical, smooth. Spores round, 2-3 $\mu$  in diam., smooth. Zygosporos formed either on the sporangiophores or on special mycelial filaments, branched sympodially. Gametes, very unequal; on unequally bifurcate filaments, the one straight, slender; the other curved, thicker. Zygosporos very variable in size, 45-150 $\mu$  in diam. Exospore brown, spiny with black points, united in plates. Endospore with simple warts. Chlamydosporos intercalary or terminal, elliptic or globose (20 x 25 $\mu$ ).

From soil: United States: Rhode Island (38)

2. *Zygorhynchus vuilleminii* Namyslowski (31)

Sporangiophores 5-8 $\mu$  broad, branched. Sporangia globose, not diffuent when young, diffuent at maturity. 30-45 $\mu$  in diam. (max. 60 $\mu$ ). Sporangia terminal, larger than other species. Columellae broader than high, ovoid, 12-30 $\mu$  broad (max. 35 $\mu$ ). Spores hyaline ellipsoid 4 $\mu$  long by 2 $\mu$  broad, often guttulate. Chlamydosporos smooth, oval or elongate, of various sizes. Zygosporos globose, epispore verrucose, brown, 40-50 $\mu$  in diam. (max. 60 $\mu$ ). Azygosporos not rare. Distinguished from *Z. moelleri* by the epispore having much smaller warts, commonly aggregated.

From soil: Canada (52), Montenegro (31)

United States: Iowa (1) (3), Louisiana (2), Maine (53), New Jersey (52) (53), Oregon (53), Rhode Island (38)

3. *Zygorhynchus moelleri* Vuillemin (27)

Turf 0.5 cm. high, grey, cottony. Sporangioophores simple or branched, and bearing one or two lateral branches, (opposite). Sporangia grey-yellow, slightly wider than long, 48 $\mu$  long by 50 $\mu$  wide. Wall not diffuent. Columellae oval and depressed, wider than long (20-30 $\mu$  high by 26-36 $\mu$  wide), wall smooth. Spores oval, 5 $\mu$  long by 3-4 $\mu$  wide (rarely 4 x 3 $\mu$ ). Zygosporos as in *Z. heterogamus* but smaller, 35 $\mu$  in diam., (extremes 20 $\mu$  and 54 $\mu$  in diam.).

From soil: Japan (45), Norway (22)

United States: Iowa (33), New York (26)

## II. THAMNIDIACEAE

5. *Thamnidium* Link 1809 (27)

Sporangiophores erect, terminated by a sporangium resembling that of the genus *Mucor*. They are formed at definite points on single or verticillate branches, which in turn are dichotomously branched and terminated by small sporangia or sporangioles. The sporangium is terminal, multisporeous, with a diffuent membrane, encrusted with calcium oxalate, and possessing a large columella. Sporangioles small, spherical containing 4-10 spores, with an encrusted membrane, persistent, not diffuent, without columellae. They are evanescent. The spores are of the same size in both sorts of sporangia; colorless, smooth. Zygosporidia naked, formed on the mycelium. Suspensors without appendages, gametes straight. Germination not known.

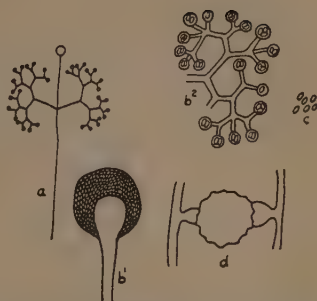


Fig. 5. *Thamnidium*. a-habit sketch; b1-primary sporangium; b2-secondary sporangia; c-spores; d-zygosporidia. (After Fischer).

\*1. *Thamnidium elegans* Link (27)

Turf 3 cm. high. Sporangiole bearing a terminal sporangium, 100-200 $\mu$  in diam., with a columella 50-70 $\mu$  wide by 62-90 $\mu$  long. The lateral branches divide in whorls and branch dichotomously. The length of the branch diminished in proportion to its forking. The first arm, from the place of insertion on the principal filament to the first fork 150-200 $\mu$  long; the arm of the first order 40-60 $\mu$ , the last are 4-6 $\mu$  long by 2 $\mu$  in diam. Sporangioles, very variable in size, up to 24 $\mu$  in diameter. The smaller have not more than 4, often only 2 or one spore. The spores are always the same size in all the sporangia, 6-8 $\mu$  wide by 8-12 $\mu$  long. Zygosporidia, according to Bainier, on the mycelium, round, black; exospore verrucose, black, endospore yellow.

From soil: England (16)

United States: Idaho (36), New York (26)

## III. PILOBOLACEAE

6. *Pilaira* van Tieghem 1875 (18)

Mycelium sunken in the substratum. Sporangioles single, thread-like, non-septate. Sporangia terminal; at first spherical; membrane cuticularized on the upper half; on the under half, delicate, thin; this latter becoming swollen upon the ripening of the spores and evanescent. Columellae large, disc-like or spherical, persistent. Zygosporidia spherical, being borne on the ends of erect copulating branches which are somewhat twisted about each other.

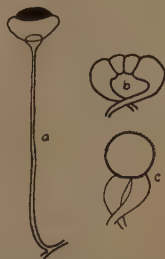


Fig. 6 *Pilaira*. a-sporangiophore; b-gametes; c-zygosporidium. (After Fischer).

\*1. *Pilaira anomala* (Cesati) Schroeter (37)

Sporangiophores only at first; at most 2 cm. high, (before they reach their full extension they appear like a *Mucor* turf,) very soon collapsing and forming a high, loose, woolly, hyaline felt on which the black sporangia appear as black points. Sporangiohores extended 10-12, even 20 cm. long, cylindric, 30-80 $\mu$  thick, without basal and sub-sporangial swellings; with colorless, thinner, shallow wavy wall, entirely empty at the time of spore maturity. Sporangia at first white, then yellow, mature black, with colorless base; wet globose, 100-250 $\mu$  in diam., dry half-round; many spored, ejaculated, sometimes nodding on the still upright conidiophore. Columellae 100-150 $\mu$  wide, 40-60 $\mu$  high, flat, half-round, or knob-shaped, smooth, colorless. Spores long-oval 8-13 $\mu$  long, 5-8 $\mu$  wide, single, colorless; in mass yellow, with colorless thin membrane. Zygosporangia at maturity black, globose or slightly oval, 120 $\mu$  long, 100 $\mu$  wide, with smooth, thick, colorless endospore and black, warty exospore. Germination with a short sporangiophore.

From soil: Holland (32)

IV. MORTIERELLACEAE

7. *Mortierella* Coemans 1873 (27)

Mycelium very thin and delicate; nutritive mycelium sunken, much branched, at times forming cysts; aerial mycelium creeping, many times anastomosing. Sporangiohores erect, with limited growth, simple or branched, very broad below, diminishing to the tip. Sporangia terminal, spherical, without columellae; membrane thin, diffuent. Spores spherical or ellipsoid. Zygosporangia spherical, covered by a thick case. Conidia formed on short side branches on the aerial mycelium, spherical, one-celled.

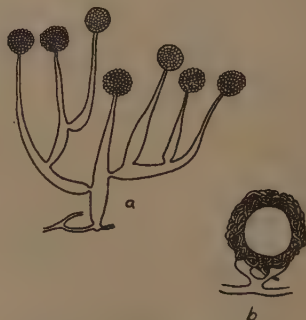


Fig. 7. *Mortierella*. a-sporangiophore; b-gametes; c-zygosporangium. (After Fischer).

KEY TO THE SPECIES OF THE GENUS MORTIERELLA

- a. Tufts white at first, later changing through grey to isabelline in color.
  1. *M. isabellina*.
- aa. Tufts always snow white.
  - b. Tufts orbicular not lamellate, sporangia not swollen at the base.
    2. *M. humicola*.
  - bb. Tufts orbicular, in layers.
    - c. Spores uniformly globose.
      3. *M. pusilla*.
    - cc. Spores of two sorts, globose 2-5 $\mu$  and elliptic 5-6 x 4-5 $\mu$  in diam.
      4. *M. subtilissima*.



\*1. *Mortierella isabellina* Oudemans (27)

Tufts elliptic, zonate, at first snow white, then pearl grey, finally isabelline (Sacc. Chrom. No. 8). Hyphae creeping, branching in forks, continuous, filled with homogeneous protoplasm; sporangiophores cylindric, slightly attenuated at the tip, continuous, 120-200 $\mu$  high, hyaline, ending in a single sporangium. Sporangia globose, 12-25 $\mu$  in diam., with hyaline wall. Spores globose, smooth, nearly hyaline when single, pale yellowish white in mass, 2.5 $\mu$  in diam. Chlamydospores submerged, globose or elliptic; smooth, hyaline, with thin membrane.

From soil: Holland (32)

\*2. *Mortierella humicola* Oudemans (27)

Tufts orbicular, not lamellate, inalterably snow-white. Creeping hyphae dichotomously branched, hyaline, continuous, at times nucleate, filled with more or less granular protoplasm. Sporangiophores cylindrical, not enlarged below, filled with a protoplasm containing rather large vacuoles, 110-150 $\mu$  in height and ending in a solitary sporangium. Sporangia globose, about 20 $\mu$  in diam., smooth, with hyaline membrane. Spores globose, smooth, up to 3 $\mu$  in diam., hyaline without trace of nucleus or oil drop.

From soil: Holland (32)

\*3. *Mortierella pusilla* Oudemans (27)

Tufts orbicular, always snow white, woolly, composed of some stages sinuous or lobed, others less wide, which are higher. Hyphae creeping, hyaline 2.5-10 $\mu$  thick, forked, filled with dense protoplasm finely granular; sporangiophores 4-6 $\mu$  wide, larger at the base, diminishing at the tip, 130-170 $\mu$  high, ending in a solitary sporangium. Sporangium globose, smooth, 24-28 $\mu$  in diam., with hyaline membrane. Spores globose, smooth, hyaline, 2-2.5 $\mu$  in diam., without trace of nucleus or vacuoles. Differs from *M. isabellina*; by the graded structure of the tufts and their inalterable white color; the content of the creeping hyphae, the form of the erect hyphae; the hyaline spores.

From soil: Holland (32)

\*4. *Mortierella subtilissima* Oudemans (27)

Tufts in all respects like those of *M. pusilla*. Creeping hyaline continuous, branched, 3-5 $\mu$  wide, filled with a homogeneous protoplasm; sporangiophores continuous, hyaline, 130-200 $\mu$  high by 2.5-3.5 $\mu$  wide, simple, cylindric, not enlarged at the base, finely attenuate at the tip, ending in a solitary sporangium. Sporangia globose, smooth, 20-26 $\mu$  in diam., with a hyaline membrane. Spores smooth, hyaline, globose 2.3 x 4.6 $\mu$  in the midst of others elliptic 5-6 x 4-5 $\mu$ .

Differs from *M. pusilla* by the homogeneous protoplasm; the finer nearly cylindric sporangiophores; the slightly smaller sporangia; the mixture of round and elliptic spores.

From soil: Holland (32)

## V. CHAETOCLADIACEAE

8. *Cunninghamella* Thaxter 1903 (27)

Mycelium white, floccose, slightly thickened, 3-6 $\mu$ , continuous when young, later becoming septate, septa disposed here and there without order. Rhizoids very tenuous. Conidiophores straight, branched. The main axis, as well as the side branches, little or not septate, terminating in spherical heads, furnished with small swellings which are the points of insertion for the conidia. Conidia spherical or oval, often with an irregular outline, the external membrane spiny with needle crystals. Chlamydospores globose, intercalary in the mycelium.

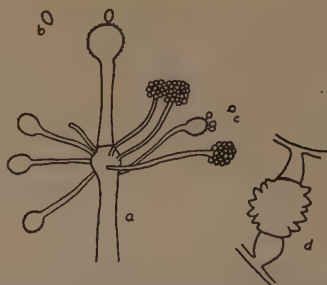


Fig. 8. *Cunninghamella*. a—conidiophore; b—primary conidium; c—secondary conidium; d—zygospore. (After Lendner).

## KEY TO THE SPECIES OF THE GENUS CUNNINGHAMELLA

- a. Spores larger: terminal—12 x 16 $\mu$ , lateral 8-10 $\mu$ ; terminal vesicle 60 $\mu$ , lateral vesicle 18-20 $\mu$  in diam.  
1. *C. elegans*.
- aa. Spores smaller: terminal—10 x 14 $\mu$ , lateral 8-10 $\mu$ ; terminal vesicle 50 $\mu$ , lateral vesicle 16 $\mu$  in diam.  
2. *C. verticillata*.

\*1. *Cunninghamella elegans* Lendner (27)

Mycelium white, very tardily ashy, is divided into two parts; the first portion in contact with the substratum is very close, and finally forms in very old cultures a cartilaginous layer which has a pseudo-parenchymatic structure. The filaments are very firm and interwoven. In the other portion, the aerial filaments are cottony and bear the organs of reproduction. Conidiophores erect, many times dichotomously branched but without septa. The tip of the conidiophore is inflated into a head very regular, round or slightly oval or pear-shaped, up to 60 $\mu$  in diam. Below this terminal head a whorl of branches variable in number occurs, each ending in a smaller rounded head (18-20 $\mu$  in diam.). Both carry the numerous conidia which differ in size according to whether the head on which they occur is terminal or lateral. On the former they are ovoid, elongated into a point at the place of insertion and measure 16 $\mu$  long by 12-14 $\mu$  wide; the upper limit is exceptionally 14 $\mu$  wide by 22 $\mu$  long. Their wall is covered with short spines. The conidia produced on the lateral heads are always smaller and more spheric (8-10 $\mu$ ). Together they give a very pale blue ash color to the culture.

From soil: Switzerland (27)

2. *Cunninghamella verticillata* Paine (33)

Colonies spreading; aerial hyphae loose, elevated, 2-4 cm. in height, somewhat silvery, much vacuolated. Conidiophores very long, 2 cm. or more, by 12 to 14 $\mu$  in thickness. Numerous lateral branches are borne at various places along the conidiophore just below the terminal vesicle, forming a number of whorls of two to six lateral branches, each terminating in

a vesicle; the conidiophore being more or less swollen at each point of attachment of the lateral branches; lateral branches not exceeding  $30\mu$  in length, their vesicles pyriform or oval, not over  $16\mu$  in diam. The terminal vesicle globose to oval, about  $50\mu$  in diam. Spores borne on the terminal vesicle ellipsoid, pointed at the attached end,  $10\mu$  by  $13$  to  $15\mu$ ; spores borne on the lateral vesicles oval, bluntly pointed at the attached end,  $8$  to  $12\mu$  in diam. All spores are finely echinulate, echinulations  $1.5$ - $3\mu$  in length.

From soil: United States: Iowa (33), Louisiana (Listed in La. Bul. 196 as *Oedocephalum*).

## II. Peronosporales

### VI. PYTHIACEAE

#### 9. *Pythium* Pringsheim 1858 (12)

Mycelium parasitic in living plants or saprophytic on insects and plants rotting in or on water, with very thin, not more than  $6\mu$  thick, often much thinner, richly paniculately branched threads, at first always one-celled, in age often with some irregularly placed cross-walls, growing intra- or intercellular, always without special haustoria; in water often forming thin, *Saprolegnia*-like turf; colorless. Sporangia not on the special conidiophores of other *Peronosporales* but partly at the end of hyphae, partly intercalary in or on the substrate, variously formed; partly thread-like, not thicker than the mycelial branches, partly spherical or lemon-shaped; the still undivided content empties into a bladder and breaks up here into zoospores, which become freed by the bursting of the bladder. In many species the sporangia

remain sitting on the mycelium, in others only in water on submerged mycelium, while they may break off as conidia, in others finally only conidia are formed. Conidia spherical or lemon-shaped, of the shape and arrangement of sporangia, germinating either as zoospores, as sporangia, or by a tube. Zoospores kidney-shaped with two cilia inserted at the side, monoplanetic, colorless, movement uniform. Sex organs partly in the interior of the substrate, partly on the hyphae growing out of it especially in water, numerous, always androgynously arranged. Oogonia small, spherical with colorless, unspotted, smooth, or warty-spiny membrane, one egg and little periplasm. Antheridia mostly club-shaped, on the end of short branches, sprouting from below the oogonium, curved secondary branches, seldom cylindrical and divided hypogynous as pieces of the oogonium bearing threads; very rarely lacking also the antheridia. Oospores single in the oogonium, spherical with large central colorless fat-drop, colorless content, with thick smooth or spiny, yellowish or gray episperm. Germination either by zoospores or tube.

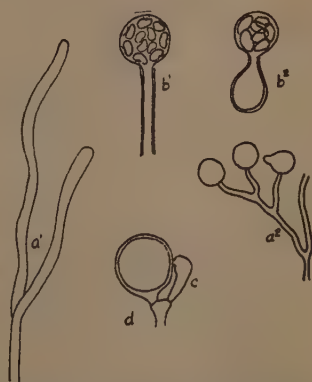


Fig. 9. *Pythium*. a1-thread-like sporangium; a2-globose sporangia; b1, b2-zoospore formation from the respective sporangia; c-antheridium; d-oogonium. (After Fischer).

## KEY TO THE SPECIES OF THE GENUS PYTHIUM

- a. Sporangia filamentous, resembling the vegetative hyphae, not separated off from the vegetative mycelium by septa, often branched and very unequal in size. Antheridia and oogonia cut off by septa. Oospores smooth.
  - 1. *P. monospermum*.
- aa. Sporangia spherical, oval, etc. (not filamentous) cut off by septa from the vegetative mycelium.
  - b. Sporangia proliferous, conidia unknown.
    - 2. *P. proliferum*.
  - bb. Sporangia not proliferous, often transformed into conidia.
    - c. Sporangia and conidia catenulate. Sexual reproduction unknown.
      - 3. *P. intermedium*.
    - cc. Sporangia and conidia not catenulate.
      - d. Sporangia rare. Conidia irregular. Oospore not filling the oogonium.
        - 4. *P. vexans*.
      - dd. Sporangia numerous, subspherical.
        - e. Oospore not filling the oogonium.
          - 5. *P. de baryanum*.
      - ee. Oospore filling the oogonium.
        - 6. *P. rostratum*.

\*1. *Pythium monospermum* Pringsheim (12)

Mycelium forming a cloud around the substratum. Hyphae irregularly branched up to  $7\mu$  in diameter, often with numerous bud-like outgrowths laterally. Sporangia single or branched, length very variable, up to 1 mm. Zoospores from a few to 40 or more. Oogonia within and outside the substratum, terminal or intercalary, or formed in the lateral buds. Antheridia one or more, club-shaped, arising from the oogonial or from a distinct hypha. Oospores smooth, completely filling oogonium, the wall of which is often difficult to define,  $12-15\mu$  in diameter, germination after a rest, which may be several months in duration, by a hypha which is quickly transformed into a sporangium.

From soil: Ireland (12)

\*2. *Pythium proliferum* de Bary (12)

Mycelium in water culture, fine. Hyphae uniform,  $4-5\mu$  broad, branching laterally and sparingly in young cultures. The origin of a lateral branch may be somewhat swollen, and rarely, fusiform swellings occur in the course of the hyphae. Sporangia terminal, spherical, rarely, oval, vacuolated, very variable in size,  $30-58\mu$  in diameter, with a short tube of discharge, rarely equalling one-fourth of the diameter of the sporangium, placed in any position, but usually opposite the stalk. After discharge, growth of the supporting hypha occurs through the emptied sporangium, or immediately below it, laterally, new sporangia being formed within the empty sporangium, or beyond it, in the first case. Zoospores large, 3 to numerous. Conidia unknown. Oogonia within and outside the substratum, terminal or often intercalary,  $19-36\mu$  in diameter. Antheridia 1-3 or more, usually more than one, from neighboring branches and, less frequently from the oogonial stalk. Oospores spherical, not filling oogonium,  $16-27\mu$



in diameter. Germination after a rest of several months, by a hypha which usually branches, the branches being short and clustered and bearing one or rarely two sporangia, sometimes, particularly in small spores, by an unbranched hypha which soon gives rise to a sporangium.

From soil: France (12), India (12)

\*3. *Pythium intermedium* de Bary (12)

Mycelium extra- and intra-matrical, forming a regular fine haze around the substratum in water culture. Hyphae very numerous, up to  $6\mu$  thick, regular, without intercalary swellings. Branching often at right angles, sometimes dichotomous, more usually lateral. In old cultures septa, with a distinct double contour, are not uncommon. The tips of all free branches usually end in spores. These measure  $18-24\mu$  in diameter, and are normally arranged in chains, up to 13 in a single chain having been observed. When ripe they fall off readily, and can germinate immediately in fresh water. Growth may continue from the hyphae immediately under the spore, which is gradually pushed to one side, as in *Phytophthora infestans*. The new hypha may arise so as to leave the lateral spore supported on a short basidium, or sometimes from a swollen part, immediately under the spore, which is very often present, and in this case the lateral spore lies sessile. Sometimes the new hypha arises further down, leaving the spore or chain of spores supported on a lateral stalk, which may itself give out branches and support new chains of spores. The chains are formed basipetally, the end spore being the oldest. The spores in the chain are usually spherical and divided from each other by short stalks, which may persist as a tiny process, rectangular in outline on the fallen spore. Sometimes, however, they are pear-shaped, in which case the narrow end of each arises directly from the spore below. The spores of a chain may germinate as sporangia or conidia, both forms occurring in the same chain. In young cultures large numbers of sporangia occur and discharge zoospores on addition of fresh water. In older ones the conidia are the chief organs found. The tube of discharge is always very short, about one-fourth of the diameter of the sporangium, and appears in any position, most frequently laterally. The conidia are often provided with thick walls, showing a distinct double contour. They can preserve their vitality if kept moist, for at least 11 months and can stand freezing. If completely air dried they soon die. Sexual organs have not been observed.

From soil: England (12), France (12), Germany (12), Ireland (12)

\*4. *Pythium vexans* de Bary (12)

The mycelium is slender, finer than that of *P. de baryanum* or *P. rotatum*, but resembling *P. intermedium*, both in thickness of the hyphae and the size of the thallus in water cultures. The hyphae taper at the ends, particularly in the lateral branches, which are given off in a very irregular manner. The branches of the secondary or tertiary order, often extend far beyond the primary hyphae, tapering into very fine filaments at the ends. This character distinguished it from any other species. Sporangia and conidia are developed on two or three days' old cultures. The former are rare. They occur both terminally and, more rarely, intercalarily, and are scarcely ever spherical or oval, but usually irregularly pear-shaped, ovoid or sub-angular. The sporangial tube of discharge is short. The sporangia and conidia measure  $17-24\mu$  in diameter, averaging about  $21\mu$ . The



conidia are filled with very dense protoplasm, vacuolation being rare. The oogonia are 22-25 $\mu$  in diam. and formed on the extra-matrical mycelium. They always arise laterally usually on short branches from the main hyphae, or sessile on the latter. The oogonium is inserted on its stalk by a broad base. The antheridia arise from the oogonial stalk and are sometimes hypogynal. Usually there is one to each oogonium, rarely two. The antheridial cell is club-shaped, or rounded, and large in relation to the oogonium. In every case seen it was closely applied to the oogonial wall, so as to fuse with the latter in a large part of its circumference. Oospores free in the oogonium, but larger in relation to it than in *P. de Baryanum*, 20-22 $\mu$  in diam., smooth, round. Germination often by giving zoospores directly, a thick tube being put out which, after growing to about the length of the diameter of the oospore, blows up at the apex into a bladder in which the contents of the oospore are divided into zoospores. In older spores (5-6 months) germination only by a branched hypha.

From soil: England (12), France (12), Ireland (12)

5. *Pythium de baryanum* Hesse (12)

Mycelium rather coarse, intra- and extra-matrical. Hyphae large, branching irregular and free, septate in old cultures. Sporangia spherical or oval, chiefly extra-matrical, terminal and intercalary; supporting hypha usually emptied of its contents for a variable distance below the sporangium and separated by septa from the full portion of the hypha and from the sporangium. Tube of discharge lateral, about the diameter of the sporangium in length. Proliferation absent. Conidia usually numerous, intra- and extra-matrical, 15-25 $\mu$  in diameter, round, oval or somewhat irregular in shape and size in old cultures, may germinate at once but more usually do so after a short rest. Oogonia usually numerous, intra- and extra-matrical, sometimes formed very easily in culture, 20-25 $\mu$  in diameter, spherical, terminal or intercalary. Antheridia up to three in number, from the same or another hypha as the oogonium, often formed close below the latter and not seldom hypogynal. Oospores 14-18 $\mu$  in diameter not filling the oogonium, spherical, smooth, germinating after a rest of some months by a branching hypha.

From soil: Europe and United States (12)

\*6. *Pythium rostratum* Butler (12)

Mycelium in water cultures large; hyphae up to 6 or even 8 $\mu$  in diam., and tapering gradually at the ends but never prolonged as fine filaments. Branching irregularly racemose. When old, the mycelium is sparingly septate. Sporangia terminal or intercalary, spherical at first, oval later, 28 $\mu$  in diameter as an average, ranging from 23-34 $\mu$ . The tube of discharge is very large and broad, usually about equal to the diameter of the sporangium and thickened about half way in its length in a characteristic fashion. It is usually lateral. Conidia rarely as frequent as sporangia and appear usually later. Oogonia usually intercalary or lateral are formed extra-matrically. They measure about 21 $\mu$  in diam. and are slightly longer than wide. They are completely filled by the oospore but the wall of the oogonium can usually be made out. Antheridia usually single, arise from the oogonial hypha. They are often extremely short. Oospores are spherical, smooth, 21 $\mu$  in diam. on the average, ranging from 12-26 $\mu$ . Germination not seen.

From soil: France (12)

## B. ASCOMYCETES

## III. Sphaeriales

## VII. CHAETOMIACEAE

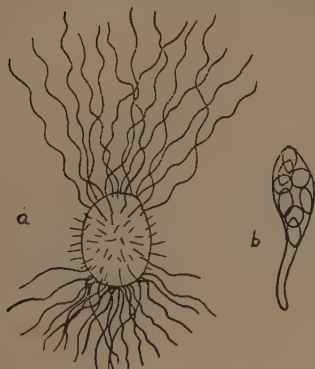
10. *Chaetomium* Kunze and Schmidt 1817 (34)

Fig. 10. *Chaetomium*. a-perithecium;  
b-ascus with ascospores.

Perithecia superficial, thin-membranous, with an apical tuft or bristles and usually with an ostium; asci club-shaped, evanescent; spores simple, hyaline to dark brown, more or less compressed.

KEY TO THE SPECIES OF THE GENUS  
CHAETOMIUM

- a. Apical hairs all simple.
  - b. Hairs flexuous, numerous, 500-700 $\mu$  long, olivaceous.
    - 1. *C. globosum*.
  - bb. Hairs contorted into loops or spirals.
    - c. Alternate loops in opposite directions.
      - 2. *C. crispatum*.
    - c. Spirally coiled.
      - d. Hairs irregularly and spirally twisted at the tip.
        - 3. *C. cochliodes*.
      - dd. Hairs more or less regularly coiled at tip.
        - 4. *C. bostrychodes*.
  - aa. Hairs all or partly branched.
    - b. Hairs up to 500 $\mu$  in length, deeply incrustated.
      - 5. *C. indicum*.
    - bb. Hairs up to 375 $\mu$  in length, smooth or slightly incrustated.
      - 6. *C. funicola*.

1. *Chaetomium globosum* Kunze (34)

Syn. *Chaetomium olivaceum* Cooke and Ellis

Perithecia scattered or gregarious, broadly ovoid or ellipsoid, often pointed at the base, 250-300 x 200-250 $\mu$ , in fresh condition olivaceous, but in dry specimens dark-brown and membranaceous, thickly and evenly clothed with slender, flexuous hairs; apical hairs somewhat coarser than the others, simple, sparingly septate, minutely scabrous, 3-4 $\mu$  thick, often 700 $\mu$  long, in the fresh condition pale olivaceous, in dry condition light brown; asci oblong-clavate, the spore bearing part 35-40 x 12 $\mu$ ; spores yellow-brown, globose ellipsoid, slightly apiculate at both ends, 9-12 x 8-9 $\mu$ .

From soil: United States: New Jersey (52) (53), New York (26)

2. *Chaetomium crispatum* Fuckel (34)

Perithecia more or less gregarious, broadly ovoid or sub-globose, reaching a height of 400 $\mu$ , membranaceous, dark-brown, thickly clothed with hairs; lateral and basal hairs smooth, septate, slender, pale-brown; apical setae forming a dense, black, spherical mass, 700 $\mu$  in diam. above the peri-

thecium, rigid, densely and minutely encrusted, light brown and distinctly septate at the base, gradually becoming darker toward the tip which is dark brown,  $11\mu$  thick, indistinctly septate, and irregularly contorted into 6-8 loops, alternate loops being in opposite directions; asci stipitate, the spore bearing part  $82-100 \times 10\mu$ ; paraphyses simple, elongate-clavate; spores 1 seriate, globose, or globose ovoid, apiculate at both ends,  $12-13 \times 9-11\mu$ , sub-hyaline or fuscous at maturity.

From soil: Japan (45)

United States: Louisiana (2)

\*3. *Chaetomium cochliodes* Palliser (34)

Perithecia scattered or gregarious, broadly ovoid to sub-globose,  $300-400\mu$  in diameter, thin, membranaceous, dark-brown, thickly clothed with hairs; lateral and basal hairs pale-brown, septate, slender, not exceeding  $4\mu$  in thickness even at the base, and gradually tapering toward the end; apical hairs extremely flexuous, almost from the base, at the end becoming irregularly spirally curved, usually smooth but occasionally minutely scabrous at the base, numerous, often forming a densely interwoven mass extending  $700\mu$  above the perithecium, spores sub-hyaline to pale brown, broadly ovoid to sub-globose, sometimes scarcely apiculate at the ends  $9-11 \times 8-10\mu$ .

From soil: United States: California (53), Hawaii (53), New Jersey (51) (52)

4. *Chaetomium bostrychodes* Zopf. (13)

Steel gray. Perithecia of medium size, extremely variable in shape, broadly ovate, globose or nearly cylindrical, generally with a bluntly pointed base,  $340 \times 220\mu$  ( $168-350 \times 131-230$ ), frequently provided with black, straight or re-curved cirrhi. Lateral hairs not numerous, encrusted, clearly and evenly septate, tapering, at base dark olive-brown and about  $3.8\mu$  in thickness, at tips pale yellow or hyaline, frequently collapsed. Terminal hairs encrusted and roughened with spine-like projections throughout, at base straight or very slightly flexed, dark olive-brown to black and about  $4\mu$  in thickness, slightly less colored at tips, always more or less spirally coiled but in this respect extremely varied. In the type either regularly coiled with seldom more than 5-7 convolutions which diminish almost imperceptibly in diameter toward the extremity, or irregularly coiled with two or three loose, irregular convolutions; in either case irregularly septate, producing along the convolutions one or more branches which in turn are septate and spirally coiled. Asci short, stout, club-shaped, 8 spored,  $50 \times 12\mu$ , *pars sporif.*  $24\mu$ . Spores when young greenish, hyaline, with granular contents, when mature pale with olive-brown tint, oval to nearly spherical, clearly or obscurely apiculate, or rounded at both ends, frequently with an elliptical, refractive area abreast of each end, a characteristic observed only in this species,  $7.4 \times 6\mu$  ( $6.4-8 \times 5.6-6.4$ ), when seen edgewise, compressed,  $4.8\mu$  broad.

From soil: United States: Iowa (33)

5. *Chaetomium indicum* Corda (13)

Black. Perithecia small, globose to verruciform,  $180$  by  $160\mu$  ( $105-200$  by  $101-175$ ), firmly attached to the substratum by dark olive-brown to black

rhizoids. Lateral hairs comparatively few, rather rigid, septate, tapering to a blunt point or drawn out into a long, hyaline, collapsed tip, at base dark olive-brown to black, and about  $5.3\mu$  in thickness. Terminal hairs of two types which can be best clearly distinguished by studying the perithecium at different ages; (a) hairs which first appear from the top of the perithecium and which do not form a dense mass, stout, dichotomously branched with branches reflexed and roughened by spine-like projections, at base dark olive-brown to black and about  $7.5\mu$  in thickness, fading only slightly or becoming hyaline at the terminal branches; (b) hairs which appear later, forming at first a tuft about the ostiole, profusely branched by narrow acute angles, branches never reflexed, alternately constricted and inflated, light olive-brown or yellow, finely roughened, terminal branchlets encrusted with clusters of acicular or prismatic crystals. Asci club-shaped, 8-spored,  $30$  by  $9.4\mu$ . Spores hyaline when young, when mature dark, rich olive-brown, ovate to lemon-shaped, slightly apiculate at one or both ends,  $5.5$  by  $4.5\mu$  ( $5.3$  to  $7$  by  $4.5$  to  $5.6\mu$ ).

From soil: United States: Louisiana

The following measurements were found for the soil culture: perithecia up to  $235\mu$  in diam.; hairs up to  $550\mu$  long; spores  $5$  to  $5.5$  by  $3.5$  to  $4.5\mu$ .

\*6. *Chaetomium funicola* Cooke (34)

Perithecia more or less scattered, small, broadly ovoid, about  $150$ - $110\mu$ , dark-brown, clothed on all sides with hairs; lateral hairs simple, comparatively short, smooth, rhizoids slender, pale-brown, flexuous; apical hairs of two kinds, simple and branched; simple hairs lanceolate, extending  $375\mu$  above the perithecium, smooth or nearly so, dark-brown or almost black at the base, gradually tapering to a point and becoming paler at the tip; branching hairs few in number or forming a mass  $180\mu$  above the perithecium, sub-hyaline or pale-brown to dark-brown, sometimes encrusted, usually smooth, with numerous ramifications, sometimes regularly dichotomous, more often irregularly branched; branches short,  $15$ - $20\mu$ , spores small, broadly obovoid, scarcely apiculate  $4.5$ - $6$  x  $3$ - $4.5\mu$ .

From soil: United States: California (53), Maine (53)

# VIII. SORDARIACEAE

## 11. *Pleurage* Fries 1849 (21)

Perithecia scattered or aggregate, superficial or sunken; membranaceous or coriaceous, without stroma; asci without an apical perforation, stretching at maturity; paraphyses ventricose or filiform-tubular, usually agglutinate and longer than the asci; spores ellipsoid, with or without primary appendages, but always having attached to them at maturity 2 or more hyaline, gelatinous, secondary appendages of variable length.

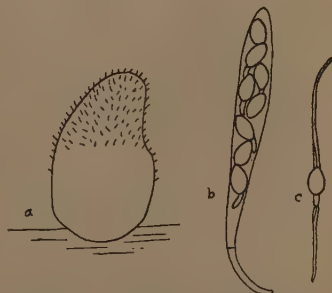


Fig. 11. *Pleurage*. a-perithecium; b-ascus; c-asospore. (After Griffiths).

\*1. *Pleurage verruculosis* Jensen (26)

Perithecia scattered or aggregated, sunken but becoming partly to entirely exposed at maturity, membranaceous to carbonaceous, black, opaque, 350-750 by 225-375 $\mu$ , pyriform to sub-globose, with straight or curved beak. Asci 4-spored, cylindrical, broadly rounded at apex and tapering below into a slender stipe, perforate, 90-150  $\times$  11-16 $\mu$ ; paraphyses filiform, slightly tapering upward, longer than the asci, septate to articulate. Spores vertically uniseriate, long ovate when young to sub-globose at maturity, obtusely pointed above and broadly rounded to truncate below, germ pore prominent, strongly tuberculate, ranging in color from hyaline when young through brown to black at maturity, 12-14  $\times$  16-18 $\mu$ , primary appendage 6-8 $\mu$  and conic shortly after migration of protoplasm from below is completed and the septum is formed, at full maturity it becomes a shrunken hyaline appendage of 3-4 $\mu$  in length; secondary appendages entirely wanting.

From soil: United States: New York (26)

12. *Sporormia* de Notaris 1849 (21)

Perithecia globose or ovoid, sunken or less frequently superficial, with papilliform to cylindric beak, membranaceous to coriaceous and sometimes slightly brittle; asci, cylindric to clavate with an internal membrane which is usually perforate at the apex; spores cylindric, 3-many-septate, usually dark-brown and opaque and surrounded by a hyaline gelatinous envelope.

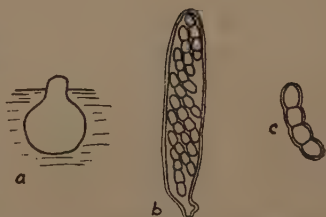


Fig. 12. *Sporormia*. a-perithecium; b-ascus; c-ascospores. (After Griffiths).

\*1. *Sporormia fasciculata* Jensen (26)

Perithecia scattered or aggregated in small clusters, sunken, with small papilliform beak projecting to the surface, later many become partly exposed, globose, then membranaceous, inclined to be brittle, black, opaque, 250-525 $\mu$  in diam. Asci 8-spored, broad clavate, broadly rounding above and rapidly contracting just below spores to form a long slender stipe 45-60  $\times$  16-30 $\mu$ ; stipe about two-fifths the length of the ascus; paraphyses absent. Sporidia fasciculate, straight or very slightly curved, 4-celled, rounded at both ends, deeply constricted and easily separating, 25-30  $\times$  4-7 $\mu$ , ranging in color from hyaline when young through light brown to dark brown, opaque.

From soil: United States: New York (26)



## IV. Hypocreales

## IX. NECTRIACEAE

13. *Neonectria* Wollenweber 1917 (61)

Perithecia in context and shape close to *Neectria*, single or gregarious, bright colored; spores long, thin, ellipsoid resembling *Mycosphaerella*, 1-many septate; conidia cylindric, referred to *Ramularia*, chlamydospores intercalary.

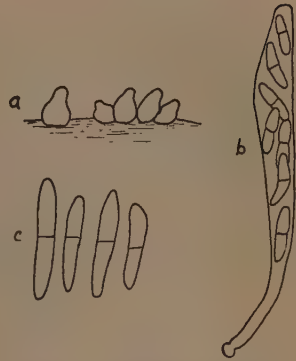


Fig. 13. *Neonectria*. a-perithecia; b-ascus with ascospores; c-conidia. (After Wollenweber).

\*1. *Neonectria ramulariae* Wollenweber (61)

Syn. *Ramularia magnusiana* (Saccardo) Lindau

Perithecia solitary or aggregated in acervuli, red, 200-300 x 170-250 $\mu$ , ovoid or globose; asci eight spored; spores in mass pale yellow, singly hyaline, long, typically ellipsoid, 1-septate 12-15 x 3.25-4 (11-20 x 3-4.5) $\mu$ , in state of germination 1-3 septate; conidia cylindric or slightly dorsiventral, sometimes semiglobose or slightly apiculate at the base, 1-septate, 20-27 x 3.5-4.5 $\mu$ , rarely non-septate, very rarely 3-septate, in tubercular sporodochia or in minute pale yellow columns, erumpent from the host epidermis; chlamydospores few, intercalary and at times formed within the walls of the conidia.

From soil: United States: Texas (56)

## C. FUNGI IMPERFECTI

## V. Sphaeropsidales

## X. SPHAERIOIDACEAE

14. *Phoma* (Fries) Desmazieres 1846 (37)

Pycnidia globose or slightly lens-shaped with a small papilla at the apex, membranaceous to leathery or almost carbonaceous, black. Spores small, egg-shaped, spindle-shaped, cylindric or almost spherical, 1-celled, hyaline, usually with two oil drops. Conidiophores thread-like, seldom short, or almost lacking, simple or sometimes forked.

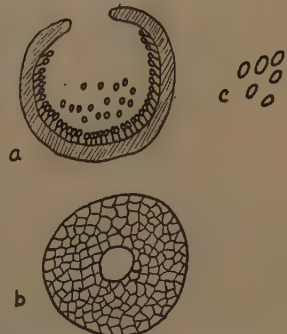


Fig. 14. *Phoma*. a, b-pycnidia; c-conidia.

1. *Phoma humicola* n. sp.

Colonies dark brown, broadly spreading, largely submerged, with little aerial hyphae. Pycnidia dark brown to black, membranous, produced slowly but abundantly, scattered, erumpent through the subicle, sub-globose to pyriform, with a short neck, from 150 by 125 $\mu$  up to 600 by 500 $\mu$  in size. Conidia oblong or bacillate, with rounded ends, hyaline, 9.5 to 12.5 $\mu$  by 3 to 4 $\mu$ .

From soil: United States: Utah

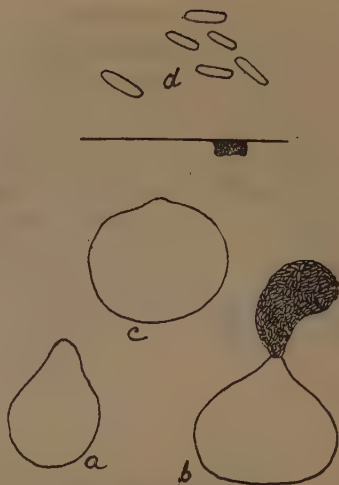


Fig. 15. *Phoma humicola*, n. sp. a, b, c-pycnidia; d-conidia.

15. *Coniothyrium* Corda 1840 (37)

Pycnidia globose or flattened below with papillate mouth, black, membranaceous to almost carbonaceous. Spores globose or ellipsoid, small, brown, one-celled. Conidiophores short, simple, or almost lacking.



Fig. 16. *Coniothyrium*. a-pycnidium; b-conidia.

## KEY TO THE SPECIES OF CONIOTHYRIUM

a. Spores small, 2.5 to 3 by 3.5 to 5.2 $\mu$ .

1. *C. fuckelii*.

aa. Spores larger, 10 to 15 by 7 to 9 $\mu$ .

2. *C. terricola*.

\*1. *Coniothyrium fuckelii* Saccardo (37) (52)

Colonies at first white, subfloccose, later becoming all black, with white aerial mycelium; reverse at first creamy, later turning black. Pycnidia submerged, scattered, black, 180 to 200 $\mu$  in diameter (Waksman (52) gives 240 to 350 $\mu$ ), depressed, spherical, with scarcely apparent, slightly protruded ostiolar papilla; spores very numerous, brown, elliptical, slightly apiculate at one end, 2.5 to 3.5 $\mu$  by 3 to 5.2 $\mu$ ; sporophores not visible.

From soil: United States: New Jersey (52) (53)

2. *Coniothyrium terricola*, n. sp.

Colonies on bean agar broadly spreading, orbicular, pure white at first, later becoming smooth and steel gray. Pycnidia submerged in the mycelium, scattered, dark brown or black, mostly 100 to 200 $\mu$  in diameter, globose to sub-globose, with small ostiolar papillae; spores very numerous, dark brown, elliptical, apiculate at both ends, 10 to 15 $\mu$  long by 7 to 9 $\mu$  broad; sporophores not observed.

From soil: United States: Iowa, Louisiana

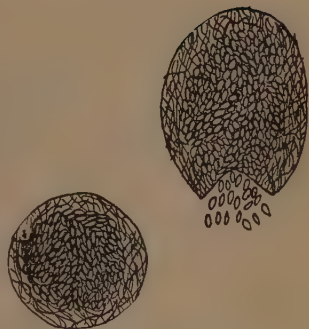


Fig. 17. *Coniothyrium terricola*, n. sp.

16. *Chaetomella* Fuckel 1869 (18)

Pycnidia superficial, sometimes short stipitate, without a mouth, but covered on the whole surface with long hairs. Spores cylindric or somewhat spindle-shaped, somewhat curved, colored. Sporophores simple or branched.

1. *Chaetomella horrida* Oudemans (26)

Mycelium creeping, from white to darkish, branched, septate; pycnidia 180 x 140 $\mu$ , superficial, scattered, ovate, without ostiole, brown, in transmitted light dark brown, with setae on all sides; setae of the old pycnidia rising high, downward black and opaque, upward lighter, dark or dilute olive, septate, when young smooth, when old slightly roughened, once or many times dichotomously branched, ultimate branches awl-shaped; spores broad elliptical, biconvex, commonly apiculate on both ends, very dilute steel-colored, 5.5-7 x 3.5-4 $\mu$ ; basidia toward the base dark, above hyaline, three times length of spores.

From soil: Holland (32)

United States: Iowa (1) (3)

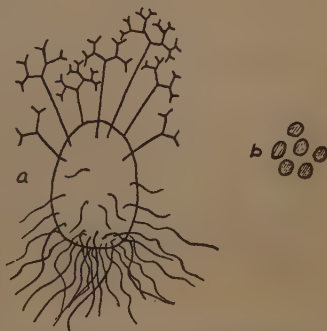


Fig. 18. *Chaetomella*. a-pycnidium; b-conidia.

## V. Moniliales

## XI. MONILIACEAE

17. *Oospora* (Wallroth) Lindau (37)

Turf spreading or cushiony, thread-like, loose or somewhat close. Hyphae creeping, septate. Fertile hyphae short, mostly simple. Conidia in regular chains, round or ovoid, hyaline or bright colored.



Fig. 19. *Oospora*. (After Lindau).

1. *Oospora lactis* (Fresenius) Lindau (37)Syn. *Oidium lactis* Fresenius

Colonies far spreading, membranaceous, velvety, pure white, often becoming a thick covering over the substratum. Hyphae simple or branched, creeping or somewhat ascending, hyaline, or variable length and breadth, mostly 6 to 12 $\mu$  broad, breaking up into irregular pieces that are to be considered as spores; spores cylindrical to ovate, often also globose to somewhat irregular in form, mostly 6 to 20 $\mu$  long.

From soil: Denmark (26), Germany (5)

United States: New Jersey (52) (53)

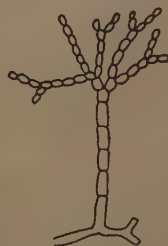
2. *Oospora variabilis* (Lindner) Lindau (37)

Forms a white turf consisting of cells of many different forms. Forming either threads of more or less long cells, which break up easily or spores with small, round buds, or yeast-like colonies.

From soil: England (16)

18. *Monilia* (Persoon) Saccardo 1880 (37)

Mycelium creeping, septate; conidiophores ascending or erect with dichotomous, racemose, or irregular branching, which is sparse or abundant; simple or branched conidial chains borne on the points of the branches or on small, blunt projections near the point. Conidia ovoid to elongate, ovoid, seldom globose, hyaline or light colored, often united by isthmus-like connecting cells.

Fig. 20. *Monilia*.  
(After Lindau).

## KEY TO THE SPECIES OF THE GENUS MONILIA

- a. Colonies white to gray.
  - b. Colonies pure white, floccose; conidia ovoid, 12 to 15 $\mu$  by 8 to 10 $\mu$ .
    - 1. *M. acremonium*.
  - bb. Colonies pure white, cottony; conidia lense-shaped, apiculate, 3 to 5 $\mu$  by 1 to 1.5 $\mu$ .
    - 2. *M. implicata*.
  - bbb. Colonies white, floccose; conidial masses red, conidia ovoid to cylindrical, 5 to 13.5 $\mu$ .
    - 3. *M. sitophila*.
- aa. Colonies yellow to brown.
  - b. Colonies ochre yellow; conidia globose to elliptical, 3 to 5 $\mu$  by 2 to 3 $\mu$ .
    - 4. *M. geophila*.
  - bb. Colonies buff brown; conidia elliptical, apiculate, 5.5 to 7.5 $\mu$  by 3 to 4 $\mu$ .
    - 5. *M. brunnea*.
- aaa. Colonies green.
  - b. Colonies cottony; conidia elliptical, apiculate, 4 to 10 $\mu$  by 2 to 5 $\mu$ .
    - 6. *M. humicola*.

\*1. *Monilia acremonium* Delacroix (37)

Colonies spreading, somewhat floccose, white. Sterile hyphae creeping, hyaline, sparsely septate, with oil drops present, 4 to 5 $\mu$  thick. Conidiophores erect, often united in bundles, with numerous septa, bearing the conidial chains terminally. Conidia ovate-pyriform, somewhat truncate at the base, united by small connecting cells, 12 to 15 $\mu$  by 8.5 to 10 $\mu$ , hyaline.

From soil: Holland (32)

2. *Monilia implicata* n. sp.

Colonies on Czapek's agar spreading, cottony to floccose, consisting of interwoven, hyaline, aerial hyphae and masses of very long, intertangled conidial chains which spread over the medium; surface pure white, reverse colorless to cream. Conidiophores prostrate, arising laterally from aerial mycelium, thickly crowded on the fertile hyphae, tapering gradually toward the apex, hyaline, 20-100 $\mu$  long. Conidial chains very long. Conidia lense-shaped, apiculate, hyaline, 3 to 4.3 $\mu$  by 1 to 1.5 $\mu$ . Characters on bean agar similar.

From soil: United States: Louisiana

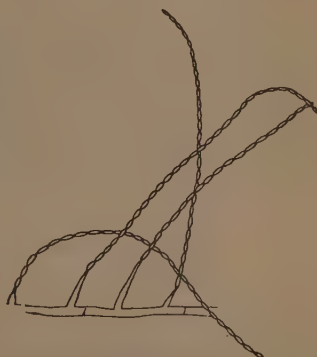


Fig. 21. *Monilia implicata*, n. sp.

\*3. *Monilia sitophila* (Mont.) Saccardo (52)

Colonies white, floccose, spreading, conidial masses red. Vegetative hyphae hyaline, freely branched, septate; surface mycelium carries short branches from which the conidial chains arise. Conidia ovoid to cylindrical, 5.2 to 13.4 $\mu$  in diam.

From soil: New Jersey (52) (53)

\*4. *Monilia geophila* Oudemans (37)

Colonies yellow to ochre yellow, composed of creeping, hyaline, branched, sparsely septate hyphae loosely floccose. Conidiophores ascending or erect, with numerous septa, toward the apex once or twice forked or irregularly branched, with few short branchlets. Conidial chains borne on the points of the branchlets, singly or in twos. Conidia at first globose, then elliptical, yellowish white, 3 to 5 $\mu$  long by 2 to 3 $\mu$ .

From soil: Holland (32)

5. *Monilia brunnea*, n. sp.

Colonies on Czapek's agar, round, not spreading, densely floccose; aerial hyphae creeping, densely interwoven, hyaline, 2.5 to 3 $\mu$  in diameter; surface pale buff brown, reverse brown. Conidiophores arise from aerial mycelium, scattered on the hyphae, 8 to 20 $\mu$  long, narrow-



Fig. 22. *Monilia brunnea*, n. sp.



ing abruptly near the apex to produce a short sterigmata-like cell which bears the conidial chains. Conidial chains often branched, short. Conidia elliptical, rather sharply pointed, smooth, light buff, 5.5 to 7.5 $\mu$  by 3 to 4 $\mu$ . Characters on bean agar similar.

From soil: United States: Louisiana

6. *Monilia humicola* Oudemans (26)

Colonies orbicular, dense, at first nearly hyaline, later entirely green; sterile hyphae creeping, when young hyaline. Conidiophores ascending or erect, yellow or green, closely septate, branched; branches alternate or nearly opposite, once or twice dichotomously branched. Conidia in short chains, elliptical when mature, both ends apiculate, greenish, 4 to 10 $\mu$  by 2 to 5 $\mu$ .

From soil: Holland (32)

United States: Louisiana, New Jersey (52) (53)

SPECIES OF MONILIA OF DOUBTFUL RELATIONSHIP

*Monilia candida* Bonorden

Poorly described. From soil: Germany (5)

*Monilia koningi* Oudemans

Listed by Dale (16) as a synonym of *Scopulariopsis rufulus* Bainier.

From soil: England (16), Holland (32)

United States: Michigan (19)

*Monilia fimicola* Cost. and Matr.

Its isolation from the soil is very uncertain; see (37)

19. *Cephalosporium* Corda 1839 (37)

Sterile hyphae creeping. Conidiophores arise as short branches of aerial hyphae, erect, non-septate, not swollen at the tip. Conidia borne singly at the tips of the conidiophores, being pushed to the side as they are formed successively, not falling away, but forming a transparent head enveloped by slime; conidia usually ovoid, hyaline or slightly colored.

KEY TO THE SPECIES OF THE GENUS  
CEPHALOSPORIUM

a. Colonies pure white, conidia 8-10 x 3.5-4.0 $\mu$ .

1. *C. curtipes*.

aa. Colonies white at first, becoming rose-colored, conidiophores up to 60 $\mu$  long.

2. *C. acremonium*.

aaa. Colonies white with light rosy center, conidiophores 100-200 $\mu$  long.

3. *C. humicola*.



Fig. 23. *Cephalosporium*. (After Corda).

1. *Cephalosporium curtipes* Sacc. (37) (52)

Colonies on Czapek's agar spreading, felty to floccose, pure white, consisting of creeping, septate, dichotomously branched hyphae, reverse colorless. Conidiophores arise as branches of aerial hyphae; short, up to  $25\mu$  long. Conidial heads round. Conidia elongate elliptical, hyaline,  $9.0$  to  $10.0\mu$  by  $3.5$  to  $4.0\mu$ .

From soil: United States: Colorado (53), Louisiana, New Jersey (52) (53), Porto Rico (53)

2. *Cephalosporium acremonium* Corda (26)

Colonies orbicular, dense, floccose, at first white, later very light rose-colored; vegetative hyphae hyaline, sparsely septate, branched. Conidiophores arise as side branches of aerial hyphae, erect, simple, non-septate,  $40$  to  $60\mu$  by  $3\mu$ . Conidia numerous, elliptical or oblong, straight or curved, nearly hyaline,  $4.0$  by  $1.0$  to  $1.5\mu$ .

From soil: England (16), Holland (32)

United States: New Jersey (52) (53)

3. *Cephalosporium humicola* Oud. (26)

Colonies orbicular, floccose, at first white, later with white margin and dilute rose-colored center; sterile hyphae septate, branched, hyaline,  $3.0$  to  $5.0\mu$  thick, intermixed with segments which appear like chlamydospores. Conidiophores erect,  $100$  to  $200\mu$  long, unbranched, non-septate; conidial heads  $20.0$  to  $26.0\mu$  in diam. Conidia globose,  $2.3$  to  $2.5\mu$ , almost hyaline.

From soil: Holland (32)

*Cephalosporium koningi* Oud (32)

This species, the isolation of which is reported from the soil by Koning in Holland (32) and Waksman from Alaska soil, (53) is believed by Lindau (37) to belong with the Mucoraceae because of the non-septate mycelium, the presence of chlamydospores, and the loose adherence of the conidia in the head.

20. *Trichoderma* (Persoon) Harz 1871 (37)

Sterile hyphae creeping, septate, forming a flat, firm turf. Conidiophores erect, arising from short, branched side-branches, branching usually opposite, not swollen at the apex and bearing terminally the conidial heads. Conidia small, mostly globose, bright colored or hyaline.

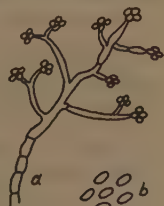


Fig. 24. *Trichoderma*.

## KEY TO THE SPECIES OF THE GENUS TRICHODERMA

a. Colonies with mature fruiting areas white.

1. *T. album*.

aa. Colonies with mature fruiting areas green.

- b. Colonies floccose, surface light green, conidia elliptical.  
2. *T. koningi*.
- bb. Colonies tufted, strict, not floccose.
- c. Surface deep green, conidia globose.  
3. *T. lignorum*.
- cc. Surface yellow to yellow-green, conidia ovoid to elliptical.  
4. *T. glaucum*.

### 1. *Trichoderma album* Preuss

This fungus produces practically no growth on Czapek's agar. A few transparent hyphae spread over the medium, visible only when held up to the light. Scattered tufts of white aerial hyphae develop, with little or no fruiting. On bean agar, colonies, thin, spreading, small, white tufts of aerial mycelium and conidiophores developing in about two weeks. Conidiophores arise from aerial mycelium, branched, up to 25 or 30 $\mu$  in length, bearing terminal heads up to 15 $\mu$  in diam. Conidia elliptical to oval, hyaline, 2.5-3.2 by 1.5-2.0 $\mu$ .

From soil: United States: Iowa, Louisiana (53), New Jersey (53)

### 2. *Trichoderma koningi* Oudemans (3)

Colonies on Czapek's agar spreading, floccose, white at first, becoming light green in four to five days; may show various shades of light green, but never becomes deep green; reverse colorless. Vegetative hyphae septate, hyaline. Conidiophores arise as branches of aerial mycelium, alternate or opposite, up to 25 $\mu$  in height by 3.0 in diameter, di- or trichotomously branched. Fruiting heads up to 10 $\mu$  in diam.; conidia oblong to elliptical, 3.2 to 4.8 $\mu$  long by 1.8 to 3.0 $\mu$  wide, smooth, hyaline.

From soil: England (16), Holland (32), Japan (45)

United States: Alaska (53), California (53), Hawaii (53), Iowa (1) (3) (53), Louisiana (2), Maine (53), Michigan (19), New Jersey (52) (53), New York (26), Oregon (53), Porto Rico (53), Rhode Island (38), Utah

### 3. *Trichoderma lignorum* (Tode) Harz. (3)

Colonies on Czapek's agar broadly spreading, hyaline; fruiting areas appear as tufts, white at first, and becoming various deep green shades with age; reverse colorless. Conidiophores arise as branches of aerial mycelium, septate, up to 70 $\mu$  in height by 3.0 $\mu$  in diam., di- or tri-chotomously branched, occasionally forming whorls. Conidial heads up to 10 $\mu$  in diam.; conidia globose to ovoid, smooth, 2.8 to 3.2 $\mu$  in diam.

From soil: Canada (53), England (16)

United States: Iowa (3), Louisiana (2) (53), Maine (53), Oregon (53), New Jersey (52) (53), New York (26), Porto Rico (53), Utah

### 4. *Trichoderma glaucum* Abbott (3)

Colonies on Czapek's agar spreading; at first only a thin, sterile, mycelial film covers the surface of the medium. White aerial mycelium develops

in five to seven days, followed in ten days by the appearance of the yellow fruiting areas, which change through shades of chartreuse yellow to citron or lime green. Vegetative mycelium hyaline, 3.0 to 6.0 $\mu$  thick, multiseptate, and freely branched; cells are often short and swollen or barrel shaped. Conidiophores arise as side branches, alternately, oppositely or irregularly branched; up to 60 $\mu$  in height by 3.0 $\mu$  in width. Conidial heads 6.5 $\mu$  to 10.0 $\mu$  in diam. Conidia smooth, hyaline, ovoid, 3.8 to 5.0 $\mu$  by 2.5 to 3.0 $\mu$ , mode 4.0 by 3.0 $\mu$ .

From soil: United States: Iowa (3)

From Goddard's description of *T. nigrovirens* (20) it is believed that he was working with *T. lignorum*.

21. *Aspergillus Micheli* 1729 (Corda 1840) (51)

Vegetative mycelium consisting of septate branching hyphae, colorless; conidial apparatus developed as stalks and heads from specialized enlarged, thick-walled hyphal cells (foot-cells) producing conidiophores as branches approximately perpendicular to the long axis of the foot-cells; conidiophores non-septate or septate, usually enlarging upward and broadening into elliptical, hemispherical, or globose fertile vesicles bearing sterigmata, either parallel and clustered in terminal groups or radiating from the entire surface; sterigmata in one series, or as a primary series, each bearing a cluster of two to several secondary sterigmata at the apex; conidia varying greatly in color, size, shape, and markings, successively cut off from the tips of the sterigmata by cross-walls, and forming unbranched chains arranged into radiate heads or packed into columnar masses; perithecia found in certain species only; sclerotia regularly found in some strains, occasionally found in others.

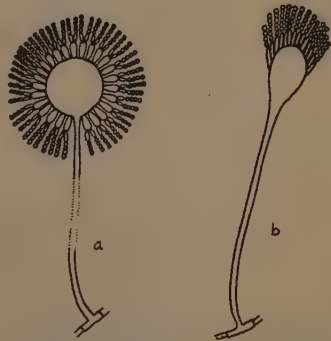


Fig. 25. *Aspergillus*. a-head with sterigmata in two series; b-head with sterigmata in one series.

KEY TO THE SPECIES OF ASPERGILLUS\*

- a. Conidial heads some shade of green.
  - b. Stalks smooth.
  - c. Vesicle cylindrical clavate, stalks coarse.
  - cc. Vesicle flask-shaped or globose, not cylindrical clavate.
  - d. Sterigmata in one series.
  - e. Conidia mostly elliptical and more than 4 $\mu$  long.
  - f. Yellow perithecia commonly found.
- 1. *A. clavatus*.
  - 2. *A. glaucus* group.  
(*A. repens*)

\* The key to this genus is adapted from that of Thom and Church (51) to include only those forms isolated from the soil.

- ff. Perithecia not found; heads enclosed in slime when ripe.
  - 3. *A. conicus*.
- ee. Conidia mostly globose, 4 $\mu$  or less; conidial heads in narrow, solid columns.
  - 4. *A. fumigatus*.
- dd. Sterigmata in two series.
  - e. Conidial chains in columns.
    - 5. *A. nidulans*.
  - ee. Conidial chains in radiate heads.
    - f. Heads blue green.
      - 6. *A. sydowi*.
    - 2f. Heads glaucous, green or yellow green.
      - 7. *A. versicolor*.
    - 3f. Heads gray green to brown green.
      - 8. *A. minutus*.
  - bb. Stalks pitted, often appearing rough, conidial heads yellow green.
    - 9. *A. flavus* group.  
(*A. humus*)
- aa. Conidial heads never green.
  - b. Stalks smooth.
    - c. Conidial heads avellaneous or brown.
      - d. Conidial heads in columns.
        - e. Conidial heads avellaneous.
          - 10. *A. terreus*.
        - ee. Conidial heads pale buff, stalks yellow, commonly with sulfur yellow sclerotia.
          - 11. *A. flavipes*.
      - 2c. Heads orange to umber, colony usually floccose.
        - 12. *A. wentii*.
      - 3c. Heads sulfur yellow.
        - 13. *A. sulphureus*.
      - 4c. Heads white, radiate.
        - d. Surface powdery, sterigmata in 2 series.
          - 14. *A. candidus*.
        - dd. Surface floccose, sterigmata in 1 series.
          - 15. *A. koningi*.
      - 5c. Heads pink, radiate.
        - A. candidus* group.
      - 6c. Heads brown to black.
        - 1. Heads chocolate brown.
          - 16. *A. luchuensis*.
        - 2. Heads black.
          - 17. *A. niger*.
  - bb. Stalks pitted or apparently rough.
    - c. Heads yellow to ochre, radiate.
      - d. Heads in ochraceous shades.



c. Sterigmata in two series.

18. *A. ochraceous*.

ee. Sterigmata in one series.

19. *A. terricola*.

dd. Heads orange to umber or brown.

20. *A. tamarii*.

1. *Aspergillus clavatus* Desm. (51)

Colonies on Czapek's agar gray green to dark green, densely matted, heavy, rapidly growing and spreading; reverse and agar more or less brownish, conidiophores with walls smooth, colorless, up to one to several millimeters in length, commonly 15-20 $\mu$  in diameter, gradually enlarged at the apex into a clavate vesicle which is fertile over an area up to 150 $\mu$  long by 20 to 25 $\mu$  in longest diam.; sterigmata 7 to 10 by 2 to 3 $\mu$ , in a single series, densely covering the fertile area, bearing long chains which frequently adhere more or less into masses; conidia elliptical, green, 2.5 to 3.4 to 4.5 $\mu$ , smooth. Perithecia not found.

From soil: United States: Iowa (1) (3), New Jersey (52) (53), Texas (53)

2. *Aspergillus glaucus* group TC (51)

Syn. *A. repens*, *A. glaucus*, *A. olivaceous*

Heads green; stalks smooth, septate; sterigmata in one series; conidia predominantly elliptical, more than 5 $\mu$  long, mostly with roughly pitted wall, rarely smooth; most species produce sessile, globose, cleistocarpic yellow to orange perithecia with membranous walls, borne above the surface of the medium and producing disk-shaped ascospores, colorless or nearly so.

Conidial heads some shade of green; color of heads frequently masked by the development of yellow to red granular incrustations of the aerial sterile hyphae, by red or yellow pigment in the substratum, and by the yellow to orange color of the perithecia. Stalks septate, smooth, either produced as very short branches of aerial mycelium or long and arising directly from the substratum; vesicles varying from only slight apical dilations of the stalk to globose; sterigmata in one series. Conidia elliptical to oval or globose, usually more than 5 $\mu$  long, rough or echinulate, sometimes smooth; green.

From soil: England (15) (16), Germany (5), Japan (45)

United States: Iowa (1) (3) (33), Louisiana (2), Michigan (19), New Jersey (52) (53)

3. *Aspergillus conicus* Blochwitz (51)

Colonies slow growing, forming a slimy, convoluted or buckled mass with submerged irregular margin, colorless, then green to dark green or almost black; reverse dark green to black; stalks and heads sometimes erect and free from slime at first, but later enveloped in a mass of greasy slime. Conidiophores short, 100 to 200 $\mu$  long; vesicles up to 20 $\mu$  in diam., fertile mostly at the apex only; sterigmata of the *A. glaucus* type, 5-10 by 2-4 $\mu$ ;

in some strains developing into secondary stalks bearing little heads; conidia elliptical, 4-6 by 3-3.5 $\mu$ , in some strains smooth, in others up to 8 $\mu$  long, becoming thick walled and rough. Perithecia not found.

From soil: England (16), Europe (51)

United States: Iowa (33)

#### 4. *Aspergillus fumigatus* Fresenius (51)

Syn. Probably *A. calytratus*

Colonies on Czapek's agar in some strains strictly velvety, in others with varying amounts of tufted aerial mycelium up to felted floccose forms, green to dark green, becoming almost black in age, spreading. Reverse and substratum, colorless to yellow. Conidiophores short, usually densely crowded, up to 300 $\mu$  (occasionally 500 $\mu$ ), by 2 to 8 $\mu$  in diam., arising directly from submerged hyphae or as branches from aerial hyphae, septate or non-septate, gradually enlarged, upward, with apical flask-shaped vesicles up to 20 to 30 $\mu$  in diam., fertile usually only on the upper half, bearing sterigmata in one series, usually 6 to 8 $\mu$  by 2 to 3 $\mu$ , crowded, closely packed, with axis roughly parallel to axis of the stalk; chains of conidia form solid columns up to 400 by 50 $\mu$ ; conidia dark green in mass, globose, 2 to 3.5 $\mu$ , mostly 2.5 to 3 $\mu$ .

From soil: Japan (45)

United States: Iowa (1) (3) (33) (53), Louisiana (2), North Dakota (53), New York (26), New Jersey (52) (53), Oregon (52), Texas (52) (53) (56), Utah

#### 5. *Aspergillus nidulans* (Eidam) Winter (51)

Colonies on Czapek's agar, white to yellowish green, or fairly deep green, velvety to more or less floccose in purely conidial areas; becoming definitely floccose when perithecia are forming, reverse and agar usually more or less reddish to dark red or reddish brown; conidiophores more or less flexuous, septate or non-septate, commonly 50 to 100 $\mu$ , less often 200 $\mu$  long, by 3 to 5 $\mu$  in diam., increasing gradually to a dome-like vesicle 7 to 15 $\mu$  in diam., bearing sterigmata in two series, parallel with axis of the stalk; primary sterigmata varying, 5 to 8 $\mu$  by 2 to 3 $\mu$ ; secondary sterigmata 7 to 10 $\mu$  by 2 to 2.5 $\mu$ ; conidia globose, up to 3 or 3.5 $\mu$ , occasionally to 4 $\mu$  in diam., smooth or rough, greenish, in parallel chains in columns up to 100 to 200 $\mu$  in length by 30 to 50 $\mu$  in diam.

Perithecia (when present) up to 200 to 300 $\mu$  in diam., surrounded by floccose white to gray mycelium; perithecial walls thin, brittle, from pink to deep red or almost black. Asci pink to purple, numerous, filling the perithecia, 8-spored; asci and purple-red ascospores varying in size.

From soil: Japan (45)

United States: Colorado (52) (53), Iowa (1) (3) (33) (53), Michigan (19) (20), New Jersey (52) (53)

#### 6. *Aspergillus sydowi* (Bainier and Sartory) Thom and Church (51)

Colonies on Czapek's agar blue green, with the bluish effect prominent, velvety, with some aerial interlacing and trailing hyphae; reverse and sub-

stratum shades of orange to red, becoming almost black; conidiophores mostly arise from submerged hyphae, up to  $500\mu$  by 4 to  $8\mu$ , colorless, smooth, thick-walled; heads radiate or globose; vesicles 12 to  $20\mu$  in diam.; sterigmata radiate in two series primary up to 7 by 2 to  $3\mu$ , secondary 7 to 10 by  $2\mu$ ; conidia globose 2.5 to  $3.5\mu$ , spinulose. No sclerotia or perithecia found.

From soil: United States: Iowa (33), Louisiana, Utah

7. *Aspergillus versicolor* (Vuillemin) Tiraboschi (51)

*A. globosus* Jensen

*A. tiraboschi*, Corbone

*A. diversicolor*, Waksman

Colonies on Czapek's agar white, passing through shades of yellow, orange-yellow, buff to pea green or sage green, with green color occasionally entirely suppressed to produce orange buff to almost flesh colored strains; reverse and agar from yellow through orange to rose or red; surface growth in some strains consisting almost entirely of conidiophores (velvety), in others showing marked development of floccose, sterile hyphae; conidiophores, when arising separately from the substratum, up to 500 or  $700\mu$  long by 5 to  $10\mu$  in diam., walls smooth, 1 to  $1.5\mu$  thick; heads becoming 100 to  $125\mu$  in diam., subglobose to globose or more or less calyptrate; vesicles 12 to  $20\mu$  in diam., occasionally globose, usually flask-shaped, fertile on the upper two-thirds, with radiating sterigmata in two series; primary sterigmata 3 to  $5\mu$  by 3 to  $10\mu$ ; secondary sterigmata 1.5 to  $2\mu$  by 5 to  $10\mu$ ; conidia globose, usually delicately roughened, 2.5 to 3 or  $4\mu$ , usually in loosely radiating chains.

From soils: England (16)

United States: Alaska (53), California (53), Iowa (3) (33), Louisiana (2), New Jersey (52) (53), New York (26), Utah

8. *Aspergillus minutus* Abbott (3)

Colonies on Czapek's agar white at first, becoming neutral gray with the appearance of fruiting areas, and changing through shades of green gray or brown gray, finally becoming dark olive to brown or almost black in some strains. Surface velvety to more or less cottony or closely floccose; reverse various shades of yellow to orange, often deepening to brown with age. Conidiophores septate, arising as short side branches of aerial mycelium, 30 to  $60\mu$  long by  $3\mu$  in diam., rarely attaining a height of  $125\mu$ ; also arise directly from substratum, up to  $250\mu$ . Heads round and radiate in young cultures, later tending toward calyptriform; vesicles small, 8 to  $18\mu$  in diam., globose; sterigmata in two series, primary 4.8 to  $6.5\mu$  by 3.5 to  $3.8\mu$ , secondary 4.8 by  $3.2\mu$ . Conidia globose verrucose, light green brown in mass, 3.2 to  $4.5\mu$  in diam., mode  $3.5\mu$ . Sclerotia or perithecia not found.

From soil: United States: Illinois, Iowa (3), Louisiana (2)

9. *Aspergillus flavus* Link (51)

Colonies on Czapek's agar widely spreading, with floccosity limited to scanty growth of a few aerial hyphae in older areas. Conidial areas rang-

ing in color from sea-foam yellow through chartreuse yellow, citron green or lime green to mignonette green; reverse and agar uncolored or yellow, or buff. Conidiophores arise separately from the substratum, 400 to 700 or 1000 $\mu$  long by 5 to 15 $\mu$  in diameter, broadening upward, walls so pitted as to appear rough or spiny with low magnification, occasionally granular, gradually enlarging upward to form a vesicle 10 to 30 or 40 $\mu$  in diameter. Heads in every colony vary from small with a few chains of conidia to large columnar masses or both mixed in the same area; small heads with small dome-like vesicles and single series of a few sterigmata up to 10 to 15 $\mu$  by 3 to 5 $\mu$ ; larger heads partly with simple sterigmata, partly with branched or double series, or with both in the same head; primary sterigmata 7 to 10 $\mu$  by 3 to 4 $\mu$ ; secondary 7 to 10 by 2.5 to 3.5 $\mu$ ; conidia pyriform to almost globose, colorless to yellow-green, sometimes almost smooth, usually rough, varying from 2 by 3 $\mu$ , 3 by 4 $\mu$ , 4 by 5 $\mu$ , or 5 by 6 $\mu$  in diam., or even larger. Sclerotia at first white, then brown, hard, parenchymatous. Perithecia not found.

From soil:

United States: California (52) (53), Hawaii (53), Iowa (1) (3), Louisiana (2) (53), New Jersey (53), Porto Rico (53), Texas (53), Utah

#### 10. *Aspergillus terreus* Thom (51)

Syn. *A. venetus* Mass. (56)

Colonies on Czapek's agar from tints of pinkish cinnamon through cinnamon to deeper brown shades in age, spreading, velvety, or in some strains developing definite floccosity or anastomosing ropes of aerial hyphae; reverse and agar from pale or bright yellow to fairly deep browns. Conidiophores to 150 or even 250 $\mu$  long by 5 to 8 $\mu$ , more or less flexuous, with walls smooth, septate or nonseptate, with apex enlarged to form a vesicle commonly 12 to 18 $\mu$ , occasionally up to 25 $\mu$  in diam., bearing sterigmata usually in two series upon its dome-like upper surface; primary sterigmata 7 to 9 $\mu$  by 2 to 2.5 $\mu$ ; secondary 5 to 7 $\mu$  by 2 to 2.5 $\mu$ ; secondary 5 to 7 $\mu$  by 2 to 2.5 $\mu$ ; closely packed. Heads becoming solid columnar masses up to 500 $\mu$  long by 50 $\mu$  in diam.; conidia elliptical to globose, 2.2 to 2.5 $\mu$  or even 3 $\mu$  in diam., smooth, in long, parallel, adherent chains. Perithecia not found.

From soil: United States: California (49) (51), Connecticut (49), Iowa (3) (33), Louisiana (2), New Jersey (49), Texas (49) (51) (56), Utah, Virginia (49)

#### 11. *Aspergillus flavipes* Group Thom and Church (51)

(*Aspergillus flavipes* Bainier and Sartory)

Colonies on Czapek's agar white at first, becoming yellowish, in some strains forming more or less abundant, closely woven, yellow masses containing many helicoid to horse-shoe shaped, thick-walled cells ("Hülle" cells). Reverse yellow to orange or brown. Heads mostly columnar or calyptriform masses, commonly persistently white, but with some strains in pale avellaneous shades to deep avellaneous. Conidiophores 300 to 500 $\mu$  by 4 to 5 $\mu$ , or up to 2 to 3 mm. in length and 8 to 10 $\mu$  in diam., smooth;

vesicles subglobose or elliptical up to 20 by 30 $\mu$ ; sterigmata in two series, primary 4 to 7 or 8 $\mu$  by 2 to 3 $\mu$ , secondary 5 to 8 by 1.5 to 2 $\mu$ ; conidia 2 to 3 $\mu$ , smooth, subglobose, colorless or nearly so.

The soil strains of this species are usually characterized by the abundant production of sulphur yellow sclerotial masses of cells.

From soil: United States: Iowa (3), Louisiana (2)

12. *Aspergillus wentii* Wehmer (51)

Colonies on Czapek's agar deeply floccose, spreading, with sterile hyphae, white or yellowish, and with heads white at first, changing through cream, cream buff, honey yellow, old gold, to light brownish olive, medal bronze, or in old cultures sometimes snuff brown (Ridgway LV, XVI, XXX, and XXIX); in some strains producing large masses of aerial mycelium which in tubes may fill the lumen 3 cm. above the substratum; reverse yellowish at first, becoming reddish brown when old; agar frequently colored yellow. Conidiophores 2 or 3 or up to 5 mm. long, 10 to 12 or 25 $\mu$  in diam., 1- to 2-septate, walls thick, smooth, enlarged at tips to vesicles varying up to 80 $\mu$  in diam.; heads large, yellow to brown, radiate; sterigmata usually in two series, primary varying greatly, 6 to 8 $\mu$ , occasionally to 15 $\mu$  by 3 to 5 $\mu$ ; secondary 6 to 8 by 3 $\mu$ ; conidia pyriform to globose, usually 4 to 5 $\mu$ , less commonly up to 5 or 6 $\mu$ ; walls often pitted or furrowed, frequently appearing smooth or nearly so. Perithecia have not been found; sclerotia have been occasionally but not uniformly produced.

From soil: Iowa (3), Louisiana (2), Oregon (50)

13. *Aspergillus sulphureus* (Fresenius) Thom and Church (51)

Colonies on Czapek's agar powdery, sulfur yellow in color, reverse brown. Conidiophores arise from aerial hyphae, up to 200 $\mu$  in length (up to 1000 $\mu$  on bean agar), stalk walls smooth. Heads loose columns of conidial chains, rarely radiate; sterigmata in two series. Conidia globose, thick-walled, smooth, 3.0 to 3.5 $\mu$ .

From soil: United States: Iowa, Louisiana

14. *Aspergillus candidus* group Thom and Church (51)

(*Aspergillus candidus* Link)

Colonies on Czapek's agar white, or becoming cream or yellowish cream in age; surface growth usually stalks and heads with scanty sterile mycelium, or anastomosing ropes of hyphae bearing short fertile stalks. Conidiophores vary with the strain, less than 500 $\mu$  long up to 1000 $\mu$  or longer by 5 $\mu$  or 10 or 20 $\mu$  in diam., walls thick, smooth; heads white, globose, radiate, varying from large globose masses 200 to 300 $\mu$  in diam., to small heads less than 100 $\mu$  in diam.; vesicles typically globose, up to 50 $\mu$  in very large heads, fertile over the whole surface; sterigmata typically in two series, primary 5 to 10 $\mu$  or even 15 to 20 $\mu$  long, secondary 5 to 8 by 2 to 2.5 or 3 $\mu$ ; conidia colorless, globose, smooth, 2.5 to 3.5 or 4 $\mu$ . Sclerotia occasionally produced.

A fungus having the general morphology of the *A. candidus* group but producing bright pink conidial heads, has been found commonly in Louisiana soils. Because of the similar morphology it was classified with



the *A. candidus* group. Some strains produce yellow sclerotia which are similar to those of *A. candidus*.

Production of bright yellow sclerotial masses have been observed in certain white strains of *A. candidus* which are found commonly in the soil.

From soil: England (15)

United States: Iowa (3), Louisiana (2), Texas (56)

15. *Aspergillus koningi* Oudemans

Colonies on Czapek's agar spreading, closely floccose, (never powdery as in *A. candidus*) vegetative hyphae creeping, septate, hyaline; surface creamy white, reverse colorless. Aerial mycelium abundant, conidiophores comparatively sparsely produced. Conidiophores arise from aerial mycelium, non-septate, hyaline, straight or flexuous, smooth; vesicles 16 to 20 $\mu$  in diam.; heads radiate; sterigmata in one series, 8 to 10 by 2.5 $\mu$ ; conidia globose, cream colored, smooth, 3 $\mu$  in diam. (Modified from the original).

From soil: United States: Iowa (3), New York (26)

This species which was isolated once from Iowa soil is not listed with the accepted species of Thom and Church, but is mentioned by Thom as an intermediate form, probably related to the *A. glaucus* group. However, it is never green. Following their key it would be placed in the *A. candidus* group. It is distinguished from *A. candidus*, however, by being floccose as opposed to powdery, and by having the sterigmata in a single series. The cream color of the surface is also different from the pure white which characterizes most of the strains of the *A. candidus* group. Because of these differences, the specific name *A. koningi* is retained here.

16. *Aspergillus luchuensis* Inui (50)

This form differs from *A. niger* in showing a single series of sterigmata 7 to 9 by 5 $\mu$ , with conidia 4 to 4.5 $\mu$  and finely roughened. Conidiophores up to 2.5 mm. by 10 to 15 $\mu$ , smooth; vesicles 20 to 40 $\mu$  in diam., showing pores or marking when sterigmata fall off; sterigmata in one series, 6 by 3 $\mu$ .

From soil: United States: Louisiana (2)

17. *Aspergillus niger* Group Thom and Church (50)

(*A. niger* van Tieghem)

(*A. fuscus*, Schumann)

Colonies on Czapek's agar rapidly growing with abundant submerged mycelium, in some strains with more or less yellow color in the hyphae, aerial hyphae usually scantily produced; reverse usually without color. Conidiophores mostly arise directly from the substratum, smooth, septate or non-septate, varying greatly in length and diameter, 200 to 400 $\mu$  by 7 to 10 $\mu$ , or several millimeters long and 20 $\mu$  in diam.; conidial heads fuscous, blackish brown, purple brown, in every shade to carbonaceous black, varying from small, almost columnar masses of a few conidial chains to the more common globose or radiate heads, up to 300, 500 or 1000 $\mu$  long; vesicles globose, commonly 20 to 50, up to 100 $\mu$  in diam.; sterigmata typically in two series, thickly covering the vesicle, primary varying greatly in length,

secondary 6 to 10 by 2 to 3 $\mu$ ; conidia globose, at first smooth, but later spinulose with coloring substance, mostly 2.5 to 4 $\mu$ , less frequently 5 $\mu$ . Globose, superficial sclerotia produced in some strains, but not common.

From soil: England (15) (16), Japan (45)

United States: California (52) (53), Iowa (1) (3) (33), Louisiana (2) (53), New Jersey (52) (53), Porto Rico (53), Rhode Island (38), Texas (53), Utah

18. - *Aspergillus ochraceus* Wilhelm (51)

Colonies on Czapek's agar ochraceous shades, consisting of conidiophores and conidial heads with little aerial mycelium. Conidiophores variable in length, commonly several millimeters, rough or pitted, yellow, bearing large, radiate conidial heads. Vesicles globose 60 to 75 $\mu$  in diam.; sterigmata in two series, primary commonly 15 to 30 $\mu$  long, although sometimes longer, secondary 7 to 10 $\mu$  by 1.5 to 2 $\mu$ ; conidia globose to elliptical, smooth or delicately spinulose, yellow, 3.5 by 5 $\mu$  or 3.5 to 4 or 4.5 $\mu$ . Orange to vinaceous or purple sclerotia commonly present.

From soil: United States: Iowa, Louisiana (2), Texas (55)

\*19. *Aspergillus terricola* Marchal (51)

Colonies on Czapek's agar with colorless submerged mycelium; conidial areas at first yellow, then golden, and finally fulvous; stalks 1 to 2 mm. high up to 20 to 25 $\mu$  in diam., septate; heads up to 500 $\mu$  in diam.; vesicles 30 to 50 $\mu$  in diam., nearly globose, and fertile over nearly the entire surface; sterigmata in one series, 8 to 12 $\mu$  by 3 to 4 $\mu$ , with long, loosely radiating conidial chains. Conidia yellow or golden, then brown, lemon-shaped, 5 to 9 by 5 to 6 $\mu$ , rough from irregularly branching ridges of yellow to brown coloring matter between the inner and outer wall. Sclerotia are occasionally found.

*Aspergillus terricola* var. *americana* Marchal (51)

Colonies on Czapek's agar from shades near yellow ochre to brown or umbrinus; aerial growth consisting of crowded conidiophores 300 to 600 $\mu$  by 6 to 8 $\mu$ , walls pitted; heads radiate; vesicles up to 20 $\mu$  in diam.; sterigmata in one series, 7 to 10 $\mu$  by 2 to 4 $\mu$ ; conidia tuberculate from the presence of color bars, ovate, from 3 by 5 $\mu$  up to 5 by 7 $\mu$  or nearly globose, usually about 5.5 $\mu$ , occasionally 5 to 8 $\mu$ .

From soil: United States: Georgia (41)

\*20. *Aspergillus tamarii* Kita (51)

Colonies on Czapek's agar spreading broadly, with vegetative hyphae mostly submerged, with fruiting areas at first colorless, then passing through orange-yellow shades to brown in old colonies, light brownish olive, buffy citrine, medal bronze, or raw umber, (Ridgway XXX, XVI, LV, III) not showing true green; reverse uncolored or occasionally pinkish; stalks arising from submerged hyphae, up to 1 to 2 mm. in length, 10 to 20 $\mu$  in diam., increasing in diameter toward the apex and passing rather abruptly into vesicles; vesicles 25 to 50 $\mu$  in diam.; heads vary greatly in size in the same

fruiting area, from more or less columnar to nearly but not complete globose and up to  $350\mu$  in diam., with radiating chains and columns of conidia; sterigmata in one series in small heads, in two series in large heads, primary commonly 7 to  $10\mu$  by 3 to  $4\mu$ , secondary 7 to  $10\mu$  by  $3\mu$ ; conidia more or less pyriform to globose, 5, 6, or up to  $8\mu$  in diameter, rough from masses of coloring matter. Sclerotia occasionally produced, usually purple or reddish purple, globose to pyriform with apex white.

From soil: United States (50)

*Species of Aspergillus of doubtful relationship; specific names not accepted by Thom and Church* (51)

### *Aspergillus calyptratus* Oudemans

According to Thom and Church (51) most of the fungi listed under this specific name are probably *A. fumigatus*.

From soil: Holland (32) from decaying wood in soil

United States: Michigan (20), New Jersey (52) (53), Texas (51)

### *Aspergillus oryzae* Cohn

The isolation of this species from the soil was reported by Takahashi (45). Since this organism is associated with the soy and sake fermentations and probably does not occur normally in soils, it was omitted from the key.

## 22. *Amblyosporium* Fresenius 1863 (37)

Sterile hyphae creeping, septate, branched. Conidiophores erect, septate, not swollen at the apex, but terminating in a number of irregular branches, on which the conidial chains are borne. Conidia long, barrel-shaped, in chains, bright-colored.

### 1. *Amblyosporium echinulatum* Oud. (26)

Colonies orbicular, gray green; vegetative hyphae hyaline, articulate, branched; fertile hyphae swollen at tip, up to  $200\mu$  high, hyaline toward the base, toward the apex with dilute gray green branches; branches basidia-like, closely and repeatedly verticillate or spirally arranged, lageniform, continuous,  $25\mu$  high; conidia catenulate, at first hyaline and globose, afterward dilute gray green and ovoid or broadly elliptical, truncate at ends, apiculate, very minutely spiny, 8 to  $12\mu$  by 6 to  $9\mu$ .

From soil: Holland (32)



Fig. 26. *Amblyosporium*. (After Lindau).

## 23. *Penicillium* Link 1809

Vegetative hyphae creeping, septate, branched; conidiophores erect, usually unbranched, septate, at the apex with a verticil of erect primary branches, each with a verticil of secondary and sometimes tertiary branches; or with a verticil of conidia-bearing cells (phialides) borne directly

on the slightly inflated apex of the conidiophores; sometimes with secondary conidiophores borne on the apex of the main conidiophore; conidia borne in chains which typically form a brush-like head, not enclosed in slime; well-differentiated foot-cells not present; conidia globose, ovoid, or elliptical, smooth or rough.

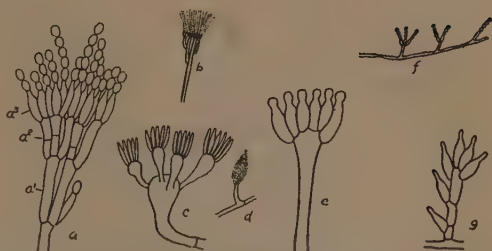


Fig. 27. *Penicillium*. a-head with three stages; a1-primary branches; a2-metulae; a3-phialides; b-habit sketch of head with two stages; c-*Aspergilloides* type of head, showing secondary conidiophores; d-habit sketch of *aspergilloides* type; e-monoverticillate head; f, g-*Scopulariopsis* type of head.

The limits of the genus *Penicillium* have been interpreted in a broad sense to include not only those species which produce well developed penicillate conidial heads, but also the monoverticillate forms. In the latter the fruiting heads in a single culture may vary from one, two, or three short chains of conidia on a single verticil of phialides to one in which several chains are aggregated into a brush-like head, and in which both metulae and phialides are present. Bainier (7) erected the genus *Scopulariopsis* to include the *Monilia*-like species which produce only a few short chains of conidia borne on a simple verticil of phialides. Wehmer's genus *Citromyces* (54) includes the species with penicillate heads in which the apex of the conidiophore is inflated and bears a single verticil of phialides. Citric acid production was also given as a generic character of this group.

Such forms are not typical members of the genus *Penicillium*, and yet it is often difficult to draw a sufficiently sharp line of distinction between the groups to justify placing them in separate genera. In the *Scopulariopsis* group, particularly, species are found with fruiting structures varying from a single short chain of conidia on a conidiophore to a more or less well developed penicillate head. From the literature it is evident that the different workers who have studied these groups are not in agreement as to their proper taxonomic position. Since the morphological differences observed by the authors did not seem great enough to justify the differentiation of these related groups into several genera, it was deemed advisable to include them as sections of the genus *Penicillium*.

It might appear that consistency in following this broad definition of *Penicillium* would make necessary the inclusion of the *Gliocladium* species in the genus, since the latter differ principally from *Penicillium* in having the conidial heads enclosed in mucus or slime. In the species examined, however, the production of slime which encloses the fruiting head was always marked, and no difficulty was experienced in determining the group to which the organism belonged.

In a previous paper (3) the opinion was expressed by one of us that both the *Aspergilloides* group of Sopp (44) and the *Citromyces* group of Wehmer (54) should be classified in the genus *Aspergillus* because of the inflated apex of the conidiophore. Thom and Church (51), however, have proposed to use the presence of a foot cell as an arbitrary character to distinguish the genus *Aspergillus* from related forms lacking this character.



Their position is accepted by the authors. *Aspergillus minutus* Abbott, which was thought to belong to the Aspergilloides group because of the short conidiophores and small vesicle, shows a well developed foot cell, and is therefore a true *Aspergillus*. The same is true of *Aspergillus humus* Abbott, which was thought to be a member of the Aspergillopsis group of Sopp. Considering the broad characterization of the *Aspergillus flavus* group as given by Thom and Church, *A. humus* becomes a member of this group species, which may or may not have branched conidiophores.

Following Biourge (9), the term phialides is used to designate the conidia-bearing cell. This organ has been called sterigma, basidium, and conidiiferous cell by different workers. No one of these terms has come into general use, however, and since there are objections to the use of the latter three, phialides was accepted as a convenient term. The supports of the phialides are termed metulae, following Wehmer. Primary branch, the meaning of which is self-evident, is used to designate the supports of the metulae in the triverticillate group.

In preparing the key presented here, Biourge's (9) main divisions of the genus were accepted as a basis. His two sub-genera *Eupenicillium* and *Monoverticillium*, and the sections within the sub-genus *Eupenicillium*, are based on natural characters, and seem to offer a satisfactory working basis. Biourge's sub-sections, however, are artificial and are based upon characters which are apt to be somewhat variable. Thom's key to specific characters was used in some instances. The principle of priority was followed in naming the sub-genera and the sections.

Sopp (44) isolated a considerable number of species of *Penicillium* from the soil, but only those which were identified were included in the key. His descriptions for the most part are rather inadequate, and do not contain sufficient information to enable separation of his species in a key.

#### KEY TO THE SPECIES OF THE GENUS *PENICILLIUM*

- a. At least two stages to the conidial fructification; phialides (conidia-bearing cells), and metulae (the supports of the phialides).

Subgenus *Eupenicillium*.

- b. Fructification typically with three stages: phialides, metulae, and primary branches.

Section *Bulliardium*.

- c. Producing white sclerotia.

1. *P. italicum*.

- cc. Not producing white sclerotia.

- d. With tendency toward coremia formation.

- e. Coremia prominent, colonies closely floccose, conidiophores rough or spiny.

2. *P. cyclospium*.

- ee. Coremia small, often lacking in soil strains, conidiophores smooth, colonies velvety.

3. *P. expansum*.

- dd. Without coremia.

- e. Colonies floccose or cottony.

- f. Colonies spreading by stolons.

4. *P. stoloniferum*.



- ff. Colonies not spreading by stolons.
  - g. Conidiophores long, up to 750 $\mu$  long, colonies blue green.
    - 5. *P. notatum*.
  - gg. Conidiophores not exceeding 150 $\mu$ .
    - h. Colonies deep green, reverse reddened.
      - 6. *P. funiculosum*.
    - hh. Colonies gray green, reverse colorless.
      - 7. *P. biforme*.
- ee. Colonies velvety or strict, not floccose.
  - f. Surface bright leaf green.
    - 8. *P. viridicatum*.
  - 2f. Surface bright green, chains from each verticil in the head in dense divergent columns.
    - 9. *P. atramentosum*.
  - 3f. Colonies gray-green or blue-green, broadly spreading.
    - g. Conidia globose, 4-5 $\mu$ , reverse white to yellowish.
      - 10. *P. roqueforti*.
    - 2g. Conidia elliptical to globose, 2 x 3.3 or 3-3.4 $\mu$ , reverse browned.
      - 3. *P. expansum*.
    - 3g. Conidia elliptical to globose 3-4 $\mu$ , reverse uncolored.
      - 11. *P. commune*.
- bb. Fructification typically with two stages only, metulae and phialides.
 

Section *Biverticillium*.

  - c. Growing colonies with prominently yellow areas, reverse yellow to orange or red.
  - d. Red or yellow sclerotial masses abundant.
    - 12. *P. luteum*.
  - e. Red or yellow sclerotial masses absent.
    - f. Colonies with sterile areas yellow and conidial areas changing through yellow shades to green.
      - g. Conidial fructification a dense column.
        - 13. *P. rubrum*.
      - 2g. Conidial fructification not a dense column.
        - h. Surface changing through yellow and pink shades to light gray-green; medium colored deep red to purple.
          - 14. *P. purpurogenum*.
        - 2h. Surface dark green, reverse bright orange.
          - 15. *P. rugulosum*.
        - 3h. Surface dark green to olive, reverse colorless to yellow or red.
          - 16. *P. duclauxi*.
        - 4h. Surface sulfur yellow, becoming olive-green.
          - 17. *P. humicola*.

- ff. Colonies developing yellow and green areas together which remain more or less permanently mixed.  
18. *P. pinophilum*.
- cc. Growing colonies with no yellow color.
  - d. Metulae inflated at the apex.
    - e. Colonies from blue green shades to olive green.  
19. *P. citrinum*.
  - dd. Metulae not inflated at the apex.
    - e. Colonies shades of clear dark green.
      - f. Colonies velvety, spreading, heads in close columns.  
20. *P. oxalicum*.
    - ff. Colonies velvety, restricted, crateriform, heads in loose columns.  
21. *P. crateriforme*.
  - ee. Colonies gray green or pale green shades.
    - f. Conidia echinulate, metulae often absent.  
22. *P. echinatum*.
    - ff. Conidia smooth, metulae always present.
      - g. Colonies cottony, mixed gray and green, conidiophores up to 300 $\mu$ .  
23. *P. chrysogenum*.
      - 2g. Colonies velvety, grayish olive, conidiophores up to 200 $\mu$ .  
24. *P. digitatum*.
      - 3g. Colonies floccose, greenish gray, conidiophores up to 50 $\mu$ .  
25. *P. intricatum*.
- aa. Conidial fructification with phialides only.
  - Subgenus *Monoverticillium*.
  - b. Apex of conidiophores inflated (sometimes only very slightly), and bearing conidial chains on phialides.
    - Section *Aspergilloides*.
    - c. With secondary conidiophores arising from the inflated apex of the main conidiophores, each apically inflated and bearing a verticil of phialides.
      - d. Colonies sulfur yellow at first, becoming yellow-green to gray-green.  
26. *P. albidum*.
    - dd. Colonies olive gray, never with yellow color.  
27. *P. acidoferum*.
    - cc. Without secondary conidiophores. (*Citromyces* Wehmer)
      - d. Colonies blue-green, conidiophores up to 400 or 500 $\mu$  long.
        - e. Colonies floccose.  
28. *P. lividum*.
      - ee. Colonies powdery, not floccose.  
29. *P. frequentans*.
      - dd. Colonies gray or olive green, conidiophores up to 75 $\mu$  long.

- e. Colonies floccose or cottony.
  - f. Conidia and elements of fructification smooth.
    - 30. *P. pfefferianum*.
  - ff. Conidia and elements of fructification rugulose.
    - 31. *P. cinerascens*.
- ee. Colonies strictly velvety, never floccose.
  - f. Surface dark grayish olive, swelling at apex of conidiophore 3-4 $\mu$  in diameter.
    - 32. *P. restrictum*.
  - ff. Surface gray-green, swelling at apex of conidiophore up to 15 $\mu$  in diameter.
    - 33. *P. glabrum*.
- bb. Apex of conidiophores not inflated, conidial chains borne directly on the conidiophores (Monilia-like) or on phialides in loose, usually divergent, heads.

Section *Scopulariopsis*.

- c. Mature colonies gray, gray-green, or green.
  - d. Reverse and substratum reddish orange to deep wine red, growing colonies with pink shades.
    - 34. *P. guttulosum*.
  - dd. Reverse and substratum colorless, growing colonies without pink shades.
    - e. Conidiophores short, up to 100 $\mu$ , conidia smooth.
      - 35. *P. decumbens*.
    - ee. Conidiophores up to 1000 $\mu$ , conidia echinulate.
      - 22. *P. echinatum*.
- cc. Mature colonies never green.
  - d. Colonies pale lilac, reverse colorless, heads loose, not columnar.
    - 36. *P. lilacinum*.
  - 2d. Colonies vinaceous to violet, reverse vinaceous to deep wine red, heads in loose columns.
    - 37. *P. vinaceum*.
  - 3d. Colonies yellowish brown to chocolate, velvety.
    - 38. *P. brevicaulis*.
  - 4d. Colonies white, becoming yellowish.
    - 39. *P. costantini*.

Subgenus *Eupenicillium* Biourge 1923 (9)

At least two stages to the conidial fructification: phialides (conidia-bearing cells), and metulae (the supports of the phialides).

Section *Bulliardium* Biourge 1920 (9)

Fructification typically with three stages: phialides, metulae, and primary branches.

1. *Penicillium italicum* Wehmer (47)

Colonies on bean agar broadly spreading, bluish green, becoming gray-green when old; reverse commonly brownish; color in medium none or

slight. Conidiophores arise either directly from the substratum or as branches of aerial hyphae, 100 to 600 $\mu$  long, averaging 250 $\mu$ . Conidial fructification up to 300 $\mu$  or more in length, usually in three stages, phialides 12 to 14 $\mu$  by 3 $\mu$ . Chains of conidia loosely divergent, long; conidia 3 to 5 $\mu$  by 2 to 4 $\mu$ , cylindrical to elliptical or slightly ovate. Numerous white sclerotia are produced upon the surface of the medium after two or three weeks growth.

From soil: United States: California (52) (53), Iowa (3), Louisiana, Oregon (53)

## 2. *Penicillium cyclopium* Westling

Colonies on Czapek's agar composed largely of coremiform masses, broadly spreading, loose; surface light blue-green; reverse light buff to reddish buff or orange. Conidiophores arise mostly as coremia, intertwined, directly from the substratum, up to 1 mm. or more in length, unbranched or dichotomously branched, each branch being also once or twice dichotomously branched near the apex. Heads long, columnar masses up to 350 $\mu$  in length; fructification usually in three stages, sometimes four, elements closely appressed, metulae oblong 8 to 10 $\mu$  by 1.8 to 2.5 $\mu$ ; phialides 4 to 6 $\mu$  by 1.5 to 2 $\mu$ . Conidiophores and elements of fructification delicately spinulose. Conidia globose to ovate, smooth, 2 to 3 $\mu$  in diameter.

Westling (57) gives 9.5 to 14 $\mu$  by 3.2 to 4.4 $\mu$  for metulae, 8 to 9 $\mu$  by 2.2 to 2.8 $\mu$  for phialides, and 2.6 to 3.2 $\mu$  for conidia.

From soil: England (16)

United States: New Jersey (52) (53), Oregon (53), Porto Rico (53), Utah

## 3. *Penicillium expansum* (Link) Thom (47)

Colonies on bean agar green or gray green, broadly spreading, becoming brown with age, floccose, often with concentric zones. Soil strains rarely produce coremia in artificial culture. Reverse brown; color in medium, none to brownish. Conidiophores either very short lateral branches of aerial hyphae or very long, 1 mm. or more, arising singly or sometimes grouped to form coremia. Conidial fructification typically in three stages, 130 to 200 $\mu$  by 50 to 60 $\mu$ , consisting of one to three primary branches bearing verticils of metulae supporting crowded whorls of phialides; phialides 8 to 10 $\mu$  by 2 to 3 $\mu$ . Conidia elliptical to globose, 2 to 3.3 $\mu$  or 3 to 3.4 $\mu$ , green.

From soil: England (16), Germany (5)

United States: California (53), Idaho (36), Iowa (1) (3), Louisiana (2), New Jersey (52) (53), New York (26), North Dakota (53), Utah

## \*4. *Penicillium stoloniferum* Thom (47)

Colonies on bean agar green or yellowish green, becoming gray-green or gray when old, floccose, spreading by aerial stolons. Conidiophores arise as short branches of aerial hyphae up to 100 $\mu$ , or arising separately 300 $\mu$  or more in length. Conidial fructification 40 to 80 $\mu$  or rarely up to 170 $\mu$  long; usually in three stages, phialides 10 by 3 $\mu$ . Conidia slightly elliptical or globose, 2.8 to 3.4 $\mu$ , smooth, yellowish-green in mass.

From soil: England (16)

\*5. *Penicillium notatum* Westling (57)

Colonies on gelatin spreading, floccose, surface bright blue-green, later becoming darker; reverse yellow. Conidiophores usually arise from submerged mycelium but also from aerial hyphae, sometimes branched, up to  $750\mu$  long by 2.8 to  $4.6\mu$  broad. Heads 45 to  $135\mu$  long; fructification in three stages (see Biourge (9); Westling's drawings indicate two); metulae 10.5 to  $14\mu$  by 3 to  $4.6\mu$ ; phialides 7 to  $8\mu$  by 2.2 to  $3\mu$ . Conidiophores and elements of fructification smooth. Conidia globose to oval, 2.6 to  $3.2\mu$  in diameter.

From soil: United States: California (53), New Jersey (52) (53)

6. *Penicillium funiculosus* Thom (47)

Colonies on bean agar deep green, broadly spreading, surface closely floccose; reverse and medium red or purple to almost black. Conidiophores arise laterally from aerial hyphae, the latter commonly in ropes; occasionally arise directly from the substratum, 20 to 80 or  $100\mu$  long. Conidial fructification in three stages, up to  $160\mu$  long, columnar; phialides 10 to  $14\mu$  by 2 to  $3\mu$ , in dense, parallel verticils. Conidia elliptical, 3 to  $4\mu$  by 2 to  $3\mu$ , green, smooth.

From soil: United States: Iowa (3), Louisiana (2)

Thom (48) classifies this species in the section Biverticillatae, although his drawings (47) show a brush in 3 stages.

7. *Penicillium bifforme* Thom (47)

Colonies on Czapek's agar broadly spreading, gray-green, becoming brownish to olive, restricted in growth, densely floccose; reverse cream; color in medium, none. Conidiophores arise from aerial mycelium, 60 to  $150\mu$  long. Conidial fructification usually in three stages, 60 to  $240\mu$  long; phialides 8 to  $13\mu$  by  $3\mu$ ; conidia elliptical to globose, 3.2 to  $3.5\mu$  by 4 to 4.3 or  $4\mu$ .

From soil: England (16)

United States: Iowa (33)

8. *Penicillium viridicatum* Westling (56)

Colonies on Czapek's or bean agar velvety, slowly spreading; surface bright leaf green, sometimes with shades of blue green; reverse colorless to buff or brown shades. Aerial sterile mycelium not abundant, usually warty and rough; colonies consist mostly of conidiophores and heads. Conidiophores usually arise from the substratum, but also from aerial mycelium, 75 to  $250\mu$  by 4 to  $6\mu$ . Heads vary from loose, almost radiate masses of chains, to loose columns. Fructification in three stages, usually with one primary branch arising laterally, a second primary branch being the prolongation of the conidiophore through the center of the head. Primary branches variable in length, 17 to  $30\mu$  by 3 to  $4\mu$ ; metulae 13 to  $20\mu$  by 3.5 to  $4\mu$ ; phialides 7.5 to  $10.5\mu$  by 2.5 to  $3\mu$ . Some heads have only metulae and phialides. Conidia smooth, globose, light green, 3 to  $4\mu$  in diameter.

Westling's (57) measurements are: conidiophores 600 by 4.4 to  $6.5\mu$ ;



metulae 10.5 to 12 $\mu$  by 4 to 5.6 $\mu$ ; phialides 8 to 9.6 $\mu$  by 3.2 to 3.5 $\mu$ ; conidia 3 to 3.8 $\mu$ .

From soil: England (16)

United States: Idaho (36), Iowa, New Jersey (52) (53), Porto Rico (53)

9. *Penicillium atramentosum* Thom (47)

Colonies on bean agar bright green, becoming brown when old; reverse uncolored or yellow; color in medium, none. Conidiophores 240 to 400 $\mu$ , averaging about 300 $\mu$  in length. Conidial fructification up to 200 $\mu$  in length, usually 100 $\mu$  or less; metulae and phialides in divergent verticils; the conidial chains from each verticil form a dense column, which diverges more or less from the other columns when old; phialides 8 to 10 $\mu$  long, closely parallel. Conidia elliptical, 3.5 to 4 $\mu$  by 2.5 to 3 $\mu$  smooth, light yellowish-green.

From soil: United States: Iowa (33), New Jersey (52) (53)

10. *Penicillium roqueforti* Thom (47)

Colonies on bean agar broadly spreading, velvety, gray-green, to clear green, becoming brownish when old; reverse colorless or cream to yellowish. color in medium, none. Conidiophores arise from submerged hyphae, 200 to 300 $\mu$  long. Conidial fructification 90 to 160 $\mu$  by 30 to 60 $\mu$ , usually appearing double by the divergence of the lowest branch, usually in three stages; metulae irregularly verticillate, bearing crowded verticils of appressed phialides, 9 to 11 $\mu$  by 2.5 $\mu$ , with long, divergent chains of conidia. Conidia bluish-green, cylindrical to globose, smooth, 4 to 5 $\mu$  in diameter.

From soil: United States: Iowa (1) (3), Maine (53)

11. *Penicillium commune* Thom (46)

Colonies on bean agar dull green, becoming brown when old, broadly spreading, with broad white growing margin composed only of conidiophores, in the older parts becoming floccose masses of inter-woven hyphae; reverse not colored. Conidiophores commonly 300 $\mu$  or less in length, sometimes up to 700 $\mu$ . Conidial fructifications commonly 100 to 200 $\mu$  in length, in three stages, compact at the base and broadening above, variously branched, with branches appressed; phialides 8 to 9 $\mu$  by 3 $\mu$ . Conidia elliptical to globose, 3 to 4 $\mu$ , smooth, green.

From soil: United States: Iowa (1) (3), New Jersey (52) (53)

Section *Biverticillium* Diereckx (17)

Fructification typically with two stages: metulae and phialides.

12. *Penicillium luteum* Zukal (47)

Colonies on bean or Czapek's agar white at first, then yellow, with few pale green conidial areas, and with abundant bright yellow to red sclerotia. Colonies commonly consist almost entirely of sclerotial areas with scant production of conidia. Reverse of colonies red. Conidiophores scantily

produced, mostly as lateral branches of aerial hyphae, 20 to 100 $\mu$  by 3 $\mu$ . Conidial fructification in two stages, up to 80 $\mu$  in length, phialides 13 to 16 $\mu$  by 3 to 4 $\mu$ . Conidia elliptical to fusiform, 2.4 by 2.3 $\mu$ , greenish, smooth.

From soil: United States: Iowa (1) (3) (53), Louisiana (53), New Jersey (52) (53); Texas (56)

13. *Penicillium rubrum* Stoll (47)

Colonies on bean agar from green through ochraceous to ochraceous red with varying conditions; consisting of green conidia with yellow mycelium in sugar media; aerial portion velvety or very closely floccose; reverse yellowish to red; coloring medium in old cultures. Conidiophores arise directly from substratum or as very short lateral branches of aerial hyphae, 15 to 30 $\mu$  by 3 to 3.5 $\mu$ , slightly swollen at the apex. Conidial fructification in two stages, usually massed into a heavy column with a broad triangular base, 100 to 200 $\mu$  in length; metulae slightly swollen at the apex; phialides 10 to 13 $\mu$  by 2 to 3 $\mu$ . Conidia elliptical to globose, 3.4 by 2 $\mu$  or 2.4 to 3.3 $\mu$ , yellowish green to green, smooth.

From soil: United States: Louisiana (2)

14. *Penicillium purpurogenum* Stoll (47)

Colonies on Czapek's agar slowly spreading, very closely floccose to almost velvety, white at first, becoming yellow to pinkish shades, and finally light gray-green; reverse and medium colored deep red to purple. Conidiophores arise from aerial mycelium, up to 100 or 300 $\mu$  long. Conidial fructifications consist of long, divergent chains, up to 100 $\mu$  long, in two stages; metulae 10 to 16 $\mu$  by 2 to 2.5 $\mu$ ; phialides 11 to 12 $\mu$  by 2.5 $\mu$ . Conidia elliptical, 3.4 to 3.8 $\mu$  by 2 to 2.5 $\mu$ , smooth, pale green.

From soil: Canada (53)

United States: Iowa (3), Louisiana (2), New Jersey (52) (53)

15. *Penicillium rugulosum* Thom (47)

Colonies on bean agar yellowish green, then green, and finally dark green; surface growth of densely crowded conidiophores with few aerial hyphae; reverse yellow to bright orange; medium slightly colored. Conidiophores arise separately or as branches of aerial hyphae, up to 200 $\mu$  by 2.5 to 3 $\mu$ . Conidial fructifications typically in two stages, (sometimes three), up to 150 $\mu$  long, conidial chains divergent; phialides 9 to 12 $\mu$  by 2 $\mu$ . Conidia 3.4 to 3.8 $\mu$  by 2.5 to 3 $\mu$ , elliptical, green, verrucose when mature.

From soil: England (15)

United States: Iowa (3), New Jersey (52) (53), Porto Rico (53)

16. *Penicillium duclauxi* Delacroix (47)

Colonies on bean agar clear deep green to olive green, often brown when old, strict, consisting of short crowded conidiophores arising for the most part singly from the substratum; reverse and medium yellow to red. Conidiophores short, 10 to 50 $\mu$  long. Conidial fructifications simple, 100 $\mu$  or 160 $\mu$  in length, consisting of a terminal whorl of phialides or with both

metulae and phialides; phialides 10 to 12 $\mu$  long. Conidia elliptical, green, smooth when young, rugulose when mature, 3.6 to 4 $\mu$  by 2 to 2.5 $\mu$ .

From soil: Japan (45)

United States: Iowa (3)

17. *Penicillium humicola* Oudemans

Colonies on Czapek's agar cottony or floccose, not broadly spreading, with bright yellow aerial mycelium and olive to gray green fruiting areas. Young colonies are bright yellow shades, becoming green as fruiting areas develop, reverse nearly colorless to orange or reddish. Colonies consist of densely woven aerial hyphae and conidiophores, the latter arising both directly from the substratum and from aerial hyphae up to 135 $\mu$  long. Heads loosely penicillate and straggling, breaking up easily; fructification in two stages, a single verticil of oblong metulae bearing the elongate and slightly pointed phialides; metulae 9.5 to 11.5 $\mu$  by 2 to 3 $\mu$ ; phialides 6.5 to 8 $\mu$  by 1 to 2 $\mu$ . Some heads have phialides only. Conidia ovoid to globose, smooth, olive green, 2 to 3 $\mu$  by 1.5 to 2 $\mu$ .

From soil: Holland (32), Japan (45)

United States: Idaho (36), Iowa, Louisiana, Michigan (19), New York (26)

18. *Penicillium pinophilum* Hedgcock (47)

Colonies on Czapek's agar cottony or very closely floccose, surface from green through various shades of mixed green, yellow, and red; reverse and medium red. Conidiophores arise as short side branches of aerial hyphae, the latter often in ropes, up to 200 $\mu$  long. Conidial fructifications in two stages up to 120 $\mu$  long, chains parallel but not in columns; metulae 10 to 16 $\mu$  by 2 to 2.5 $\mu$ ; phialides acuminate, 13 to 15 $\mu$  by 2 to 2.5 $\mu$ . Conidia elliptical, smooth, pale green or yellow, 3 to 3.6 $\mu$  by 2 $\mu$ .

From soil: United States: Iowa (1) (3), Louisiana (2), New Jersey (52) (53)

19. *Penicillium citrinum* Thom (47)

Colonies on bean agar bluish-green to clear green, becoming olive to brownish olive when old, usually with sterile white margin; reverse yellow. Aerial part of colony consists of densely standing conidiophores except in the center, where tufts of aerial hyphae arise. Conidiophores arise separately from submerged hyphae or from mycelium on the surface, usually up to 150 $\mu$  in length (rarely 300 $\mu$ ). Conidial fructifications up to 150 $\mu$  in length (rarely 300 $\mu$ ). Conidial fructifications up to 150 $\mu$  in length, in two stages; metulae 16 to 30 $\mu$  by 3 $\mu$ , enlarged at the apex to 5 $\mu$  each producing a compact verticil of phialides 6 to 7 $\mu$  by 2 to 3 $\mu$ . Conidial chains in columns, a separate column arising from each verticil of cells, so that the fructification may appear double, triple, or more complex. Conidia globose, 2.4 to 3 or 3.5 $\mu$ , green, slightly granular.

From soil: United States: Iowa (3), Louisiana (2)

20. *Penicillium oxalicum* Thom (14)

Colonies on Czapek's agar, ivy green, velvety, spreading widely with surface growth of conidiophores only; reverse pale yellow; agar uncolored

or only slightly colored; conidiophores up to  $200\mu$  by  $3.3$  to  $5.4\mu$ , enlarged to  $5\mu$  at the apex. Conidial fructifications consist of a single verticil of 2 to 3 branches (metulae)  $15$  to  $20\mu$  by  $3.5\mu$  appressed, bearing verticils of phialides  $10$  to  $14\mu$  by  $2.5$  to  $3.5\mu$ , in parallel whorls which bear conidial chains in close columns. Conidia at first cylindrical, then elliptical, from 2 by 3, 2 by 4, 2 by 5, up to 3.5 by  $5\mu$ .

From soil: United States: Iowa, New Jersey (52) (53)

## 21. *Penicillium crateriforme* n. sp.

Colonies on Czapek's agar small, round, restricted, velvety, consisting of conidiophores and heads with little aerial mycelium; surface deep dark green to dull blackish green (XLI); reverse colorless, or reddish in old cultures. Conidiophores densely crowded, arising from the substratum or from surface hyphae, up to  $250\mu$  long, mostly  $100$ - $150\mu$ . Fructification a loose column, not divergent, not densely packed up to  $225\mu$  long; in two stages, metulae oblong,  $8.5$  to  $11.5\mu$  by  $3$  to  $4\mu$ ; phialides flask-shaped,  $6.5$  to  $9.5\mu$  by  $2$  to  $3\mu$ . Conidia globose to slightly ovoid, smooth, light green,  $2.5$  to  $3\mu$ .

From soil: United States: Louisiana.

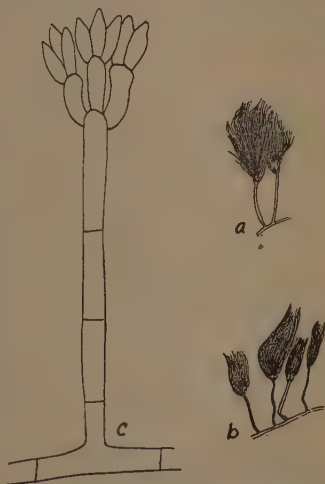


Fig. 28. *Penicillium crateriforme*, n. sp. a, b-habit sketches; c-conidiophore.

## 22. *Penicillium echinatum* Dale (36)

Colonies on Czapek's agar white to gray, gray-green, pale green, or pale bluish green, when old becoming various shades of gray and brown, spreading slowly but broadly with usually a white sterile margin throughout the growing period and slow development of colored fruit from the center outward, surface growth from velvety at the margin with center floccose to floccose out to the very edge of the colony, some strains zonate, reverse of colony at first colorless, in some strains remaining so, in others developing colors, a succession of colors appearing in series so that different strains become finally yellow, orange, orange-red, rosy, or even deep red; agar not discolored beyond the areas of immediate contact. Conidiophores either rising directly from the substratum or as branches of aerial hyphae, from very short up to  $1000\mu$  in length, or longer, slender mostly,  $2$  to  $4\mu$  in diameter, with walls smooth in some strains, slightly granular or roughened in others, or showing both conditions in the same culture; conidial fructifications variously branched from a single terminal verticil of phialides to a verticil of metulae, including the main stalk prolonged and a single branch or a whorl of branches, more rarely twice verticillate or partly so; sterigmata few in each verticil, mostly slender  $7$  to  $10\mu$  by  $2$  to  $2.5\mu$ , narrowing to a slender tube from which the conidia are formed. Conidia at first definitely elliptical,  $1$  to  $2.5\mu$  in diameter, becoming  $3$  or  $3.5\mu$  in age, con-

tinuing elliptical or becoming almost globose, either smooth or delicately roughened, or both conditions in the same strain.

From soil: England (36)

United States: Idaho (36), Iowa (33)

This species is attributed to Dale since it was so referred to by Dr. Church in private correspondence with Mr. Frederick S. Paine. The original description which was prepared by Dr. Thom from a fungus received from Miss Dale, was published without name in Pratt's paper on soil fungi (36).

This species often has monoverticillate heads as well as biverticillate. As noted in the description there are seldom more than two metulae, one a prolongation of the main stalk, and one a side branch. In the strain isolated from Iowa soil, the monoverticillate heads were predominant.

### 23. *Penicillium chrysogenum* Thom (47)

Colonies on bean or Czapek's agar gray green or mixed green and gray, becoming brownish when old, cottony to subfloccose, broadly spreading, with broad, sterile margin when young; reverse commonly yellow; medium uncolored. Conidiophores mostly arising separately, up to 300 $\mu$  long; some as short branches of aerial hyphae. Conidial fructifications 100 to 200 $\mu$  long, with one or two alternate, divergent branches; usually in two stages, but may also have three; phialides 8 by 2.5 $\mu$ . Conidia elliptical, becoming globose, 3 to 4 $\mu$ , pale green.

From soil: United States: California (52), Colorado (53), Iowa (1) (3) (33), Louisiana (53), New York (26), New Jersey (53), North Dakota (53), Porto Rico (53), Utah

### \*24. *Penicillium digitatum* Saccardo (47)

Colonies on bean agar grayish olive, aerial portion consisting only of very short conidiophores and conidia; surface grayish olive; reverse commonly brown to black. Conidiophores arise directly from the substratum 30 to 100 $\mu$  by 4 to 5 $\mu$ , usually very short. Conidial fructifications a few tangled conidial chains up to 160 $\mu$  in length, in two stages; phialides 13 to 16 $\mu$  by 3 to 4 $\mu$ . Conidia cylindrical to almost globose, 4 to 7 $\mu$  by 6 to 8 $\mu$ , often uneven in size and shape in the same chain.

From soil: United States: New Jersey (52) (53)

### 25. *Penicillium intricatum* Thom (47)

Colonies on bean agar gray, greenish gray, when old gray or smoky, floccose; reverse and medium colorless to yellow. Conidiophores sometimes terminal, more commonly branches of aerial hyphae, 30 to 50 $\mu$  long. Conidial fructifications 50 to 140 $\mu$  long, becoming longer in old cultures, usually in two stages, often only simple verticils of phialides or 1-3 verticils on divergent metulae; phialides 8 to 10 $\mu$  by 2 to 2.5 $\mu$ . Conidial chains more or less divergent, frequently aggregated into a loose column. Conidia elliptical or globose, hyaline or pale green, smooth, granular within 2.5 to 3 $\mu$ .

From soil: England (15)

United States: Connecticut (47), Iowa (1) (3), New Jersey (53)



Subgenus *Monoverticillium* Biourge (9)

Conidial fructification with phialides only.

Section *Aspergilloides* Sopp (44)

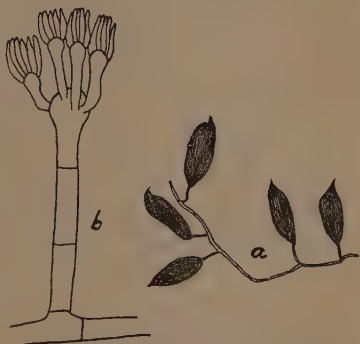
Apex of conidiophores inflated (sometimes only very slightly), and bearing conidial chains on phialides.

26. *Penicillium albidum* Sopp

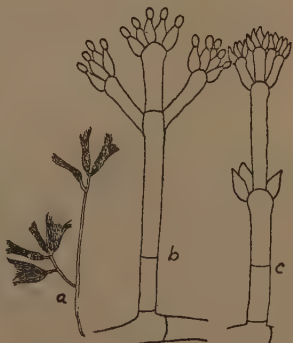
Colonies on bean agar floccose, not broadly spreading, white at first, becoming bright yellow, then yellowish green as fruiting areas develop, and finally olive to brownish olive in old cultures; reverse various shades of orange. Aerial hyphae abundant, yellowish green in color, warty, often in ropes, from which the conidiophores arise as branches. Conidiophores septate, yellowish green, warty, inflated at the apex to form a vesicle, from which several secondary, apically inflated conidiophores arise. These bear the conidial heads. Fructification a single verticil of appressed phialides which bear the conidial chains, the whole a dense column up to 75 $\mu$  long when young, but becoming long, dense, calyptriform masses in old cultures, up to 250 $\mu$  long. Conidiophores 20 to 55 $\mu$  long; primary vesicle usually about 5 $\mu$  in diameter; secondary conidiophores 6 to 8 $\mu$  long by 2 $\mu$  thick; secondary vesicle 3 $\mu$  in diameter; phialides flask-shaped, 4.5 to 7 $\mu$  by 0.7 to 1.5 $\mu$ . Conidia globose, light yellowish green, thick walled, finely roughened under oil, 2.5 to 3.2 $\mu$  in diameter. Sopp (44) gives 3-4 $\mu$  for conidia.

From soil: Norway (44)

United States: Louisiana

Fig. 29. *Penicillium albidum*. a-habit sketch; b-conidiophore, showing secondary conidiophores.27. *Penicillium acidoferum* Sopp

Colonies on Czapek's or bean agar slowly spreading, cottony or closely floccose; surface olive gray; reverse orange buff; pigment diffuses into the medium. Aerial hyphae abundant, smooth; hyaline, creeping. Conidiophores arise from aerial hyphae as short side branches up to 40 or 50 $\mu$  long, unbranched, or once or twice branched at the apex; each branch bears a terminal head of conidial chains, which are loosely columnar, up to 75 or 100 $\mu$  long, and usually with 5 to 10 chains in each head; apex of conidiophores slightly inflated; phialides 6.5 to 8.5 $\mu$  long by 2 to 3 $\mu$  thick. Conidia globose, light green, delicately rugulose under oil,

Fig. 30. *Penicillium acidoferum*. a-habit sketch; b, c-conidiophores.

2.5 to 3.5 $\mu$  in diameter. Elements of conidial fructification pitted. Sopp (44) gives 3-4 $\mu$  for conidia.

From soil: Norway (44)

United States: Utah

This species is closely related to *Penicillium rubens* Biourge (9). *P. acidoferum* has smaller conidia and phialides and the elements of the fructification are rough, whereas those of *P. rubens* are smooth.

\*28. *Penicillium lividum* Westling (57)

Colonies show abundant development of floccose mycelium, with the colony surrounded by a rather broad margin; surface blue-green, becoming darker, and finally dark brown in age; reverse white to yellowish. Conidiophores arise from submerged mycelium, up to 450 $\mu$  long, smooth, usually unbranched, although sometimes with one side branch; apex inflated up to 6.5 $\mu$  in diameter; phialides numerous, 9 to 12 $\mu$  by 2 to 2.4 $\mu$ . Heads 45 to 150 $\mu$  long. Conidia oval to ovoid, 2.5 to 4 by 2.2 to 2.6 or up to 3.6 $\mu$ .

From soil: Canada (53), England (16)

United States: New Jersey (52) (53)

\*29. *Penicillium frequentans* Westling (57)

Colonies blue-green to dark blue green or olive green, deepening with age to dark brown green; velvety or powdery, not floccose or felty; reverse yellow to reddish yellow; colony surrounded by a sterile white margin. Conidiophores arise from aerial hyphae, up to 500 $\mu$  long; often short, 60 to 225 $\mu$ ; enlarging at the apex to form a swelling 3 to 4.5 $\mu$  in diameter. Heads 45 to 115 $\mu$  long. The conidial chains are borne on phialides only, 8 to 11.5 $\mu$  by 2.2 to 3.2 $\mu$ . Conidia globose, smooth or slightly verrucose, 2.6 to 4 $\mu$  in diameter.

From soil: United States: New Jersey (53)

\*30. *Penicillium pfefferianum* Wehmer (37)

Colonies loose, cottony, spreading; surface green to gray-green, with growth of superficial hyphae. Sterile hyphae hyaline, septate, ascending, branched. Conidiophores simple or branched, up to 70 $\mu$  long by 3 $\mu$  in width, and inflated at the apex to form a swelling 4 to 8 $\mu$  in diameter. Conidial chains long, borne on a verticil of phialides which cover the swelling, pointed at the apex, 9 to 14 by 2 to 4 $\mu$ . Conidia globose, smooth, hyaline, 2.3 to 3.8 $\mu$  in diameter, light green to gray or brownish in mass.

From soil: United States: Iowa (52) (53), New Jersey (52) (53), North Dakota (53), Oregon (52) (53)

31. *Penicillium cinerascens* Biourge (9)

Colonies on Czapek's agar round, slowly spreading, closely floccose; surface light gray green; reverse colorless. On bean agar, surface gray to blue gray; reverse colorless. \* Aerial hyphae often in ropes, from which the conidiophores arise as short side branches, unbranched, pitted, slightly inflated at the apex, 6 to 60 $\mu$  long by 2 to 3 $\mu$  in width, mode 10 to 25 $\mu$ . The heads are loose columns of five to ten chains up to 100 $\mu$  long. The apex of the conidiophores bears a single verticil of flask-shaped phialides, pitted or echinulate, 5 to 7 $\mu$  by 1.8 to 2.2 $\mu$ . Conidia globose, light green, smooth or very delicately rugulose, 2 to 2.5 $\mu$  in diameter.

From soil: United States: Utah

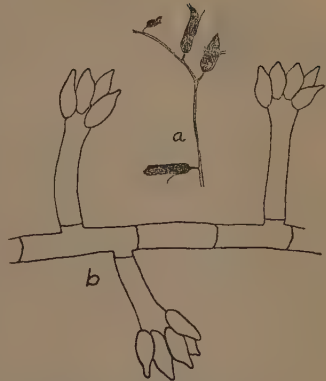


Fig. 31. *Penicillium cinerascens*. a-habit sketch; b-conidiophores.

32. *Penicillium restrictum* n. sp.

Colonies on Czapek's agar small, round, restricted, raised, velvety; surface deep to dark grayish olive; reverse colorless. Conidiophores arise from aerial hyphae, hyaline, smooth, unbranched, 10 to 50 $\mu$  by 2 to 2.5 $\mu$ , rarely reaching a height of 75 $\mu$ . The apex of the conidiophore is usually only very slightly inflated, from 0.5 to 1 $\mu$  larger than the diameter of the conidiophore, and bears a verticil of crowded, flask-shaped phialides, 5 to 7 $\mu$  by 2 to 3.2 $\mu$ . The heads are loose columns of five to ten chains up to 85 $\mu$  long. Conidia globose, delicately echinulate under oil, 2 to 2.5 $\mu$  in diameter.

From soil: United States: Louisiana

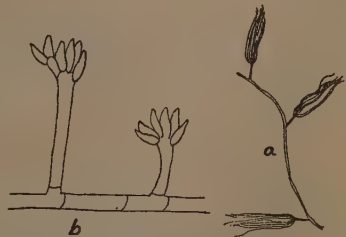


Fig. 32. *Penicillium restrictum*, n. sp. a-habit sketch; b-conidiophores.

\*33. *Penicillium glabrum* Wehmer (37) and (56)

Syn. *Citromyces glaber* Wehmer

Colonies smooth, velvety, spreading, with a narrow sterile margin; surface at first bright gray green, becoming darker, and deepening to brown green with age; reverse brown. Conidiophores inflated at the apex to form a swelling up to 15 $\mu$  in diameter; phialides 7.5 to 11.6 or 12 $\mu$  by 2 to 2.8 $\mu$ . Conidia globose, smooth, 2 to 3 $\mu$  in diameter.

Neither Lindau nor Westling give the length of the conidiophores. The species is similar to *P. pfefferianum*, however, and the conidiophores are probably less than 75 $\mu$  long.

From soil: England (16)

United States: New Jersey (52) (53)

Section *Scopulariopsis* Bainier (7)

Apex of conidiophores not inflated, conidial chains borne directly on the conidiophores (Monilia-like) or on phialides in loose, usually divergent, heads.

34. *Penicillium guttulosum* n. sp.

Colonies on Czapek's agar small, not spreading, felty surface; from pink in some strains to pinkish-gray or greenish-gray; often studded with exuded droplets of wine-red moisture; reverse orange, reddish brown to deep wine red; color in medium same, pigment rapidly diffusing and coloring the medium a deep reddish-brown to wine red. Conidiophores arise from aerial hyphae, 15 to 50 $\mu$  long by 1.5 to 2 $\mu$ , and bearing at the apex a single verticil of crowded phialides. Heads a few, short, divergent chain up to 25 or 30 $\mu$  long; phialides flask-shaped, 7 to 8.5 $\mu$  by 2 to 2.5 $\mu$ . Conidia globose to ovoid, light green, delicately echinulate, 2.5 to 3.5 $\mu$  in diameter.

From soil: United States: Utah

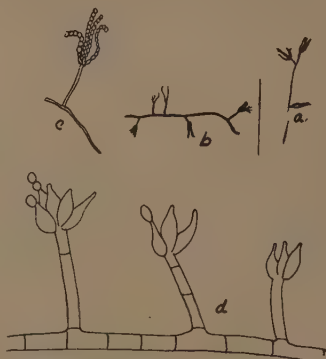


Fig. 33. *Penicillium guttulosum*, n. sp. a, b, c-habit sketches; d-conidiophores.

35. *Penicillium decumbens* Thom (47)

Colonies on potato agar white to gray, gray-green, ultimately yellowish brown, green in cultures with cane sugar; reverse colorless, surface growth consisting of trailing or stolon-like hyphae sparsely developed and so close to the substratum as to appear only as fertile hyphae, bearing the conidiophores as short branches 20 to 100 $\mu$  in length, in old cultures with dense tufts of sterile secondary mycelium scattered over the surface; conidial fructifications consisting of single verticils of phialides 7 to 9 by 2 to 3 $\mu$ , bearing conidial chains first in loose columns up to 100 $\mu$  in length, but soon enveloped and broken up in the drops of fluid secreted abundantly from the mycelium. Conidia globose 2.5 to 3 $\mu$ , smooth, pale green, then brownish in mass.

From soil: United States: Colorado (53), Iowa (1), New Jersey (52) (53), Oregon (53)

36. *Penicillium lilacinum* Thom (47)

Colonies on bean agar white to pale lilac, more or less loosely floccose with hyphae branched, septate, ascending, 3 $\mu$  in diameter, producing conidial masses upon very short branches irregularly distributed; reverse not discolored. Heads up to 100 $\mu$  in length, consisting of solitary, sessile phialides or verticils of phialides, or short branches bearing one, two, or three verticils of phialides with long, tangled chains of conidia. Phialides flask-

shaped, divergent at the apices, acuminate, 7 to 10 $\mu$  long. Conidia elliptical, smooth, pale lilac, 2.5 to 3 by 2 $\mu$ .

From soil: England (16)

United States: Iowa (3), Louisiana (2), New Jersey (52) (53)

37. *Penicillium vinaceum* n. sp.

Colonies on Czapek's agar spreading, floccose, consisting of densely interwoven, and sometimes roped hyphae. Surface white at first, becoming vinaceous to lavender, in some strains and in others vinaceous gray or green as fruiting areas develop; reverse white at first, becoming vinaceous or deep wine red in some strains. Conidiophores arise from aerial mycelium or directly from the substratum, 20 to 110 $\mu$  long, mode 50 to 75 $\mu$ . Conidial heads a few chains in loose columns up to 60 $\mu$  long when young, but becoming up to 200 $\mu$  long when mature. Chains of conidia borne on a single verticil of phialides, 6.5 to 11.5 $\mu$  by 2 to 2.5 $\mu$ . Conidia ovoid, to elliptical, smooth, echinulate 2.5 to 3.5 $\mu$  in diameter.



Fig. 34. *Penicillium vinaceum*, n. sp. a-habit sketch; b-conidiophores.

From soil: United States: Utah

38. *Penicillium brevicaulis* Saccardo (47)

Syn: *Scopulariopsis brevicaulis* Saccardo

*S. rufulus* Bainier

*S. repens* Bainier

Colonies on sugar gelatin white at first, then yellowish brown or chocolate, consisting of short closely crowded conidiophores making powdery areas overgrown by loose trailing floccose hyphae and ropes of hyphae, with broadly spreading, indeterminate margin. Conidiophores short, 10 to 30 $\mu$  long, arising directly from the submerged hyphae, or numerous and irregularly borne as lateral and perpendicular branches of trailing aerial hyphae and ropes of hyphae. Conidial fructifications either simple chains terminating unbranched or sparingly branched conidiophores in young colonies, or verticillately and irregularly twice verticillately branching systems bearing numerous divergent chains, often 150 $\mu$  in length in old colonies. Phialides continuous with conidiophores, 12 to 15 $\mu$  by 4 $\mu$ , tapering at the apex. Conidia somewhat pear-shaped, slightly tuberculate at the apex, with broad base, 6.5 to 7.5 $\mu$  by 7.5 to 9 $\mu$ , in mass light brown to chocolate, smooth at first, then with thick tuberculate walls.

The isolation of this species from the soil under the name *Scopulariopsis brevicaulis* was reported by Waksman in New Jersey (52) (53) and Texas (53); Dale (16) isolated *S. repens* Bainier and *S. rufulus* Bainier, but was not positive of her identifications, and it is probable that both should be included as *Penicillium brevicaulis*.

\*39. *Penicillium costantini* Bainier (7)

Syn. *Scopulariopsis costantini* Bainier

Colonies white, then dirty white, sometimes yellowish, in old cultures more or less dirty golden yellow. Conidial heads 30 to 50 $\mu$  long, irregular;



fructification without metulae, or rather deformed, 7 to  $8\mu$  by 3 to  $5\mu$ ; phialides 9 to 16 or 20 to  $25\mu$  by 2.5 to  $5\mu$ ; conidia 6 to  $8\mu$  by 3.5 to  $6.5\mu$ .

From soil: England (16)

### Species of *Penicillium* of Doubtful Relationship

With the information at hand regarding the following species of *Penicillium*, it was impossible to separate them with certainty in the key. They are therefore listed separately as species of doubtful relationship.

1. *Penicillium geophilum* Oudemans

From soil: Holland (32)

2. *Penicillium desiscens* Oudemans

From soil: United States: California (53), New Jersey (52) (53); New York (26), Maine (53), Porto Rico (53), Texas (53)

3. *Penicillium silvaticum* Oudemans

From soil: Holland (32)

4. *Penicillium terrestre* Jensen

From soil: England (16)  
United States (26)

5. *Penicillium candidum* Link

From soil: Japan (45)  
United States: Michigan (20)

24. *Spicaria* Harting 1871

Conidiophores erect, septate, usually freely branched, branching often in whorls but also irregular; each branchlet bears a terminal fructification composed of a verticil of divergent metulae on which are borne a verticil of divergent phialides; heads divergent and seldom penicillate; conidial chains usually long; conidia hyaline, round ovoid, elliptical, or elongate.



Fig. 35. *Spicaria*.  
(After Lindau).

### KEY TO THE SPECIES OF THE GENUS SPICARIA

a. Conidiophores smooth.

b. Colonies gray green, conidia elliptical, 6 to 12 by 4 to  $6\mu$ .

1. *S. silvatica*.

2b. Colonies gray, sometimes with rosy tints, conidia globose, 2 to  $3\mu$ .

2. *S. simplicissima*.

3b. Colonies brownish olive to olive buff.

3. *S. divaricata*.

4b. Colonies bright violet or lavender.

4. *S. violacea*.

aa. Conidiophores spiny or echinulate, colonies white.

5. *S. elegans*.

\*1. *Spicaria silvatica* Oudemans (26)

Colonies orbicular, light gray green; vegetative hyphae creeping, hyaline, septate, with forked branching. Conidiophores erect, sparsely branched; branches alternate; variable in length, simple or forked at the tip, bearing phialides 20 to 25 $\mu$  long, cylindrical and somewhat curving. Conidia in long chains, elliptical or oblong, hyaline, smooth, 6 to 12 $\mu$  by 4 to 6 $\mu$ .

From soil: Holland (32)

United States: Rhode Island (38)

2. *Spicaria simplicissima* Oud. (26)

Colonies orbicular, with alternating zones of cream yellow sterile mycelium and gray fruiting areas, occasionally with rosy tints. Conidiophores arise from aerial mycelium, 40 $\mu$  long, septate, usually unbranched, bearing metulae and phialides or phialides only; phialides 8 to 12 $\mu$  long, verticillate. Conidia in short chains, globose, 2 to 3 $\mu$  in diameter.

From soil: Holland (32)

United States: Iowa (3), New York (26)

3. *Spicaria divaricata* (Thom) n. comb.

Syn. *Penicillium divaricatum* Thom (47)

Colonies on Czapek's agar broadly spreading, low growing, felty, with scattered, floccose aerial mycelium; surface olive, olive buff or brownish olive, never true green; reverse colorless. Conidiophores arise from aerial or submerged mycelium, freely and irregularly branched, conidiophores up to 325 $\mu$  long. The conidial fructification is typically in two stages, the branches of the conidiophore bearing a terminal verticil of divergent metulae, with divergent phialides. Metulae extremely variable in length, phialides 10 to 25 $\mu$  by 2.5 to 4 $\mu$ . Conidial chains very long, divergent, seldom more than five or six in a head. Conidia elliptical, smooth, 4.5 to 6 $\mu$  by 2.5 to 4 $\mu$ .

From soil: United States: Illinois, Iowa, Utah

4. *Spicaria violacea* Abbott (3)

Colonies on Czapek's agar floccose, spreading, surface white at first, becoming bright lavender or violet when mature; reverse colorless. Aerial mycelium abundant, consisting of a dense network of interwoven hyphae. Conidiophores arise as branches of aerial mycelium, erect, up to 100 $\mu$  long, usually once or twice branched, but often short and unbranched. Conidial chains very long, up to 700 $\mu$  or more in length; fructification a divergent head with both metulae and phialides or with phialides only; phialides 6.5 by 2 $\mu$ . Conidia elliptical, smooth, hyaline, 3 to 3.5 $\mu$  by 2 to 2.5 $\mu$ .

From soil: United States: Iowa (3), Louisiana (2)

5. *Spicaria elegans* (Corda) (37)

Colonies somewhat spreading, white, velvety; vegetative hyphae creeping, hyaline, septate; conidiophores erect, septate, with two to four circles of opposite or three to four verticillate branches; branches short, fusiform, each divided at the tip into a verticil of three branchlets; branchlets lageniform, swollen at the tip; conidia ovate-fusiform, united to form long chains,  $4.5\mu$  by  $3.5$  to  $4\mu$ , hyaline.

From soil: United States: Iowa (33)

\*6. *Spicaria decumbens* Oudemans

Poorly described.

From soil: Holland (32)

25. *Gliocladium* Corda 1840

Conidiophores erect, simple or branched, septate, producing at the apex a fructification composed of successive verticils of primary branches, secondary branches, metulae, and phialides, or in some cases without secondary branches; primary branches often arise laterally on the conidiophores below the main head; conidial heads enveloped in slime, conidia in chains, or held together in a mass of slime in which chains are not distinguishable.

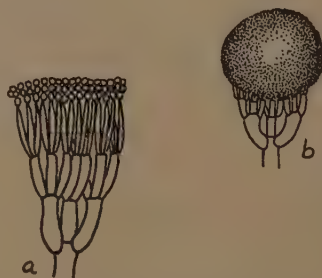


Fig. 86. *Gliocladium*. (After Lindau).

## KEY TO THE SPECIES OF THE GENUS GLIOCLADIUM

- a. Mature colonies never green.
  - b. Mature colonies pure white to cream.
    - 1. *G. penicilloides*.
  - bb. Mature colonies pink or rose shades.
    - 2. *G. roseum*.
- aa. Mature colonies green.
  - b. Conidia definitely in chains which form dense columns.
    - 3. *G. catenulatum*.
  - bb. Conidial heads round, enveloped in slime in which chains are not distinguishable.
    - c. Colonies pure white, with leaf green fruiting areas.
      - 4. *G. fimbriatum*.
    - cc. Colonies always dark green, never white
      - d. Slime production very abundant, conidiophores hyaline, rough.
        - 5. *G. deliquescens*.
      - dd. Slime production not abundant, conidiophores olivaceous, smooth.
        - 6. *G. atrum*.

1. *Gliocladium penicilloides* Corda (3)

Colonies on Czapek's agar broadly spreading, floccose, surface pure white to pale cream in fruiting areas; reverse colorless. Aerial mycelium abundant, from which the conidiophores arise as side branches, erect, septate, 50 to 100 $\mu$  long by 3 $\mu$  in diameter, pitted or rough. Fruiting heads enveloped in slime, columnar. Fructification in three stages, primary branches 15 to 25 $\mu$  long, by 3.2 $\mu$  in diameter; metulae 10 to 15 $\mu$  by 2.5 $\mu$ ; phialides 10 to 14 $\mu$  by 1.5 $\mu$ . Conidia in definite chains, elongate elliptical to bacillate, smooth, hyaline, 3.5 to 4 $\mu$  by 2 $\mu$ .

From soil: England (16)

United States: Iowa (3), Louisiana

\*2. *Gliocladium roseum* (Link) Thom (47)

Syn. *Penicillium roseum* Link

Colonies on potato agar loose floccose, with simple hyphae and ropes of hyphae, surface white to pink or salmon in fruiting areas; reverse colorless. Produces dense irregular pinkish masses or sclerotia up to 1 mm. or more in diameter in old cultures. Conidiophores borne as branches of aerial hyphae, 45 to 125 $\mu$  long. Conidial fructification enclosed in slime, up to 140 $\mu$  long, in two or three stages, phialides 12 to 17 $\mu$  by 2 to 3 $\mu$  bearing conidia in gelatinous balls or masses. Conidia colorless (pink or rosy in mass), elliptical, 5 to 7 $\mu$  by 3 to 5 $\mu$ , slightly apiculate, smooth, appearing granular within.

From soil: Japan (45)

3. *Gliocladium catenulatum* n. sp.

Colonies on Czapek's agar pure white, spreading, floccose, becoming olive green to bright green in the center as fruiting areas develop, and clear dark green in old cultures; fruiting areas are usually confined to center of colony and one or two concentric zones separated by sterile mycelium; reverse colorless to yellowish. Aerial mycelium abundant, simple or in ropes, from which the conidiophores arise as branches. Conidiophores often once and sometimes twice branched, coarse, pitted or rough, 50 to 125 $\mu$  long. Heads are composed of conidial chains in long, close columns, enveloped in slime, up to 150 $\mu$  long. Fructification in three stages, elements of fructification pitted or rough; primary branches 15 to 20 $\mu$  by 3.5 to 4 $\mu$ ; metulae 7 to 9 by 15 to 25 $\mu$ ; phialides 10 to 20 $\mu$  long. Conidia elliptical, smooth, pale green, 4 to 7.5 $\mu$  by 3 to 4 $\mu$ .

From soil: United States: Utah



FIG. 37. *Gliocladium catenulatum*, n. sp.  
a-habit sketch; b-conidiophore.

4. *Gliocladium fimbriatum* n. sp.

Colonies on Czapek's agar broadly spreading, orbicular, pure white at first, with zones of dark leaf green fruiting areas appearing near the center of the colony. Conidiophores arise from aerial hyphae, smooth, up to  $25\mu$  long; several from one point, stolon-like hyphae usually present at point of origin. Heads enveloped in round balls of slime in which chains are not distinguishable; fructification in two stages, with divergent branchlets or metulae which bear elongate flask-shaped, appressed phialides, or with conidia borne directly on a few finger-like phialides which arise irregularly from the conidiophore; in most heads one or more branchlets arise laterally from the conidiophore some distance below the main head; metulae elongate, extremely variable in size, phialides usually 10 to  $20\mu$  long, from flask-shaped to irregular elongate. Conidia elliptical or elongate, ovoid, smooth, pale green,  $6.5$  to  $9.5\mu$  by  $2.5$  to  $4\mu$ .

From soil: United States: Iowa, Louisiana

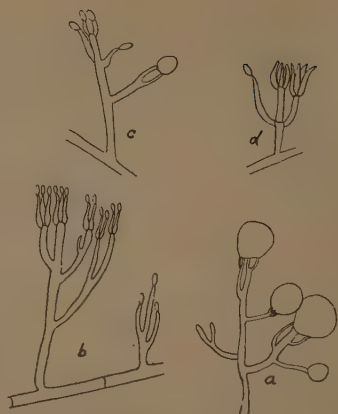


Fig. 38. *Gliocladium fimbriatum*, n. sp. a-habit sketch; b, c, d-conidiophore.

5. *Gliocladium delinquens* Sopp

Growth not abundant on Czapek's agar. On bean agar, broadly spreading, producing a thin, transparent growth of sterile hyphae over the entire medium, from which the dark green fruiting areas soon develop; surface deep, dark green to blackish green; reverse colorless. Aerial mycelium scant, colony consisting almost entirely of conidiophores and slimy heads. Conidiophores arise from submerged and surface hyphae, several from one point; both aerial and submerged stolons present at these points; conidiophores 100 to  $225\mu$  by 8 to  $10\mu$ . Fructification typically in four stages, consisting of three to five primary branches arising from the apex of the conidiophore; these bear a verticil of secondary branches, and these verticils of metulae; phialides closely crowded on the metulae, club shaped; primary and secondary branches and metulae elongate oblong, slightly inflated at the apex. Primary branches 15 to  $20\mu$  by 3 to  $3.5\mu$ ; secondary branches 13 to  $15\mu$  by  $3\mu$ ; metulae 8 to  $10\mu$  by 1.5 to  $2\mu$ ; phialides



Fig. 39. *Gliocladium delinquens*, n. sp. a-habit sketch; b-conidiophore.



6 to  $8\mu$  by 1 to  $1.5\mu$ . Conidia elliptical, greenish, smooth, granular within, 3 to  $3.8\mu$  by 2 to  $2.5\mu$ . Hyphae, conidiophores, and elements of fructification coarse and pitted, or rough. Slime production very abundant, usually enveloping the entire colony.

From soil: Norway (44)

United States: Louisiana

6. *Gliocladium atrum* n. sp.

Colonies on Czapek's brown green, small, slowly spreading, largely submerged; aerial mycelium olivaceous, scanty, aerial growth consisting mostly of conidiophores; colonies moist with slime which envelops the heads. On bean agar considerable aerial mycelium is produced. Conidiophores arise mostly from submerged hyphae, olivaceous, thick walled, smooth, septate, often slightly flexuous, 75 to  $300\mu$  by 3 to  $4\mu$ . Conidial heads enveloped in slime, round, chains not distinguishable; fructification typically in three stages, sometimes in two or four. Primary branches oblong, 8.5 to  $9.5\mu$  by 3 to  $3.5\mu$ ; metulae oblong, 7.5 to 9.5 by  $3\mu$ ; phialides flask-shaped, 7.5 to 10 by 1.5 to  $2.5\mu$ . Conidia oval to ovoid, smooth, light green to almost hyaline, 2.5 to  $4\mu$  by 2 to  $2.5\mu$ .

From soil: United States: Louisiana

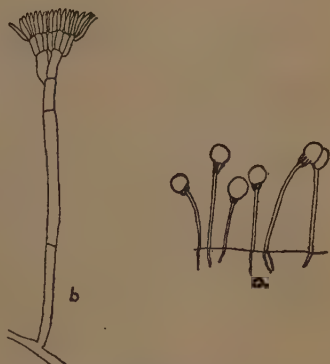


Fig. 40. *Gliocladium atrum*, n. sp. a-habit sketch; b-conidiophore.

There is sufficient color in the conidiophores of this fungus to place it with the Dematiaceae. However, the morphological structure is that of the genus *Gliocladium*, and it was placed in this genus because of its evident relationship to the other species included in this group.

26. *Sporotrichum* Link (37)

Hyphae creeping, irregularly branched, but never in whorls, branches repeatedly branched. Conidiophores not formed or only as projections from the side branchlets. Conidia borne laterally and terminally on the hyphae or the branches, usually very numerous, sessile or on small sterigmata, ovoid or globose, hyaline or brightly colored, usually very small.



Fig. 41. *Sporotrichum*. (After Lindau).

KEY TO THE SPECIES OF THE GENUS SPOROTRICHUM

- a. Colonies reddish, conidia ovoid,  $4\mu$  by  $3\mu$ .
  - 1. *S. roseum*.
- aa. Colonies white, conidia elliptical,  $9.5\mu$  by  $5.5$  to  $7.5\mu$ .
  - 2. *S. pruinoseum*.

1. *Sporotrichum roseum* Link (37)

Colonies broadly spreading, red. Hyphae creeping, sparsely septate. Conidiophores arising as short side branches, unbranched with 2 to 3 sterigmata-like branches at the tip. Conidia terminal, ovoid, reddish, 4 by 3 $\mu$ , with an oil drop.

From soil: England (16)

United States: Louisiana, North Dakota (53), New Jersey (53), Texas (53)

2. *Sporotrichum pruinosum* n. sp.

Colonies on Czapek's agar pure white broadly spreading, cottony; on bean agar low growing, dusty or powdery; reverse colorless; consisting of branched, hyaline, often roughened, aerial hyphae from which the conidiophores arise as branches; sterile hyphae often roped, up to 10 $\mu$  thick. Conidiophores freely branched, oppositely or irregularly up to 25 $\mu$  long, bearing terminal conidia, oval or lemon-shaped, 9.5-13.5 x 6-10 $\mu$ ; appearing grayish.

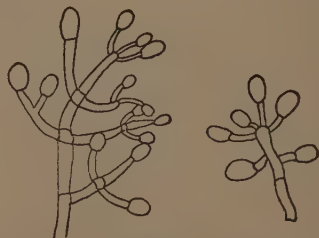


Fig. 42. *Sporotrichum pruinosum*, n. sp.

From soil: United States: Iowa, Louisiana

*Sporotrichum pulviniforme* Thom

Listed by Waksman (52) who was not sure of the identification. The species was not included in his later paper (53).

27. *Monosporium* Bonorden 1851 (37)

Sterile hyphae creeping, septate, branched, forming a turf. Conidiophores erect, septate or non-septate, branched in a tree-like form, usually with two or more erect or horizontal branches occurring above one another, which may often branch again at the tip into two or three short branches. Conidia on the final branchlets of the conidiophore, terminal, seldom one borne laterally and sessile, or less frequently with a short stipe, hyaline or bright colored, smooth, one-celled, thin walled, usually rather large, ovoid or spherical.



Fig. 43. *Monosporium*, n. sp.

Differs from *Sporotrichum* by the erect conidiophores; from *Verticillium* by the complete absence of whorled branches; from *Sepedonium* by the smooth spores.

## KEY TO THE SPECIES OF THE GENUS MONOSPORIUM

a. Spores small, 3 x 2 $\mu$ .

*M. silvaticum*.

aa. Spores large, 5-6 x 3 $\mu$ .

*M. acuminatum* var. *terrestre*.

\*1. *Monosporium silvaticum* Oudemans (37)

Colonies orbicular, white; vegetative hyphae creeping, branched, hyaline; conidiophores erect, continuous, hyaline, dendroidly branched, with ultimate branches commonly two, rarely three-forked. Conidia single, acrogenous, obovate, 3 by 2 $\mu$ .

From soil: Holland (32)

\*2. *Monosporium acuminatum* var. *terrestre* Saccardo (37)

Colony spreading, white. Conidiophores erect, slightly septate with tree-like branching, branches erect, simple or forked, pointed. Conidia long, hyaline, 5 to 6 $\mu$  by 3 $\mu$ .

Its presence in soil is uncertain. Reported from moist soil by Saccardo.

28. *Botrytis Micheli* 1729 (37)

Hyphae creeping. Conidiophores simple or frequently branching in an irregular dendroid arrangement, erect. Branches either thin or thicker and narrowing to a point, truncate or with swollen warts on the tips or toothed comb-like. Conidia frequently on the tips of the branches, but not uniformly in heads, globose, ellipsoid or long, hyaline or bright colored, one-celled. The genus contains a large number of forms which have nothing in common one with the other except a certain superficial similarity. In some species the genus forms mycelial sclerotia which are very similar to those of the genus *Sclerotinia*.

KEY TO THE SPECIES OF THE GENUS BOTRYTIS

- a. Conidia small, 2.5-3 x 3-4 $\mu$ .
  - 1. *Botrytis terrestris*.
- aa. Conidia larger, 9-12 x 6.5-10 $\mu$ .
  - 2. *Botrytis cinerea*.

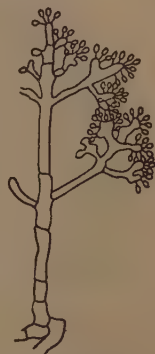


Fig. 44. *Botrytis*.  
(After Landau).

\*1. *Botrytis terrestris* Jensen (26)

Colonies at first white, later gray; sterile hyphae creeping, hyaline, branched, septate, 1.5-3 $\mu$  in diameter; conidiophores erect, ascending, septate, branched, 2-3.5 $\mu$  in diameter, 50-200 $\mu$  high; primary and secondary branches verticillate, dichotomous, or alternate; conidia produced on the ends of the branches, forming more or less compact triangular clusters that average 20-25 $\mu$ , obovate, somewhat angled, uniform 2.5-3 by 3-4 $\mu$ , hyaline to light gray. Clusters of conidia separate very easily.

From soil: United States: New York (26)

2. *Botrytis cinerea* Persoon (37)

Colonies diffuse, gray, gray green, dark olive green to brown black, seldom brown or reddish green, dusty from the conidia, loose or dense, up to 2 mm. high; conidiophores erect, unbranched or seldom branched, septate, 11 to 23 $\mu$  thick, wall blackish-brown, toward the tip almost hyaline, with several (3 and more) projections at the tip from which the conidia

are formed singly on very fine warts. The point of the conidiophore grows between the warts, thereby pressing them back, usually some distance from one another, and they become lateral. The conidia stand so thickly on the projections that thick heads are produced which soon fall off. Conidia ovoid or elliptical to almost globose, finely apiculate at the base, 9 to 12 $\mu$  by 6.5 to 10 $\mu$ , with almost hyaline, slightly brownish wall.

From soil: England (15) (16), Japan (45), Switzerland (26)

United States: Louisiana, New Jersey (52) (53), Porto Rico (52)

The following species of *Botrytis* are listed by Jensen (26) as soil fungi, but they were isolated from leaves and sticks in contact with the soil, and not from the soil itself:

*Botrytis fulva* Link

*Botrytis dichotoma* Corda

*Botrytis geophila* Bonorden

*Botrytis epigaea* Link

*Botrytis purpureospadicea* (Fuckel) Lindau

29. *Sepedonium* Link 1809 (37)

Hyphae creeping loosely branched, carrying conidia at the tips of the final branchlets. Conidia single or 2-3, terminal, globose, warty, or ovoid, hyaline or bright colored. Sometimes simple ovoid conidia occur on the upper branches of the conidiophore. Then as in *Stephanoma* the warty spores are known as chlamydo spores, the small egg-shaped forms as conidia.



Fig. 45. *Sepedonium*. (After Lindau).

\*1. *Sepedonium chrysospermum* (Bulliard) Fries (37)

Syn: *Ozonium croceum* Persoon

Hyphae widespread forming a thick white, then golden-yellow turf creeping in and on the substrate, septate, branched, tolerably thick, bearing lateral short simple or clustered branches on the tips of which the spores occur. Chlamydo spores single, acrogenous, formed in large numbers, globose, warted, yellow or golden yellow, 13-17 $\mu$  in diameter, with rather thick wall.

From soil: England (16)

United States: New Jersey (53)

30. *Pachybasium* Saccardo 1885 (37)

Hyphae forming a turf, creeping, septate, branched. Conidiophores erect, branched, primary branches sterile ending in long curved, thin hyphal tips, secondary branches alternating or standing in almost opposite whorls, on the ends of which occur many short flask-shaped terminal branchlets on which are formed the conidia. Conidia globose or elongate, hyaline or bright colored.



Fig. 46. *Pachybasium*. (After Lindau). a-colony habit; b-conidiophore; c-conidia.

\*1. *Pachybasium hamatum* (Bonorden) Saccardo (37)

Turf more or less extended, white or straw-colored, formed of floccose, curved, septate, branched hyphae, up to  $16\mu$ , in chains. Conidiophores erect, simple or many times dichotomously divided, septate and the primary branches sterile, ending in long tips bending back and forth. Side branches short, alternating or whorled, forming at their tip 2-4 flask-shaped branchlets which are elliptical in outline and narrowed to a sterigma-like tip  $1-2\mu$  long, the whole being  $10-12\mu$  long and  $7\mu$  broad. Conidia single, terminal on the branchlet, ovoid,  $7\mu$  long by  $4.3\mu$  wide, hyaline or slightly colored.

From soil: United States: Michigan (20)

31. *Verticillium* Nees 1817 (37)

Sterile hyphae creeping, septate, branched, hyaline or lightly colored. Conidiophores erect, septate, branched. Branches of the first order whorled, opposite or alternate; branches of the second order whorled, dichotomous or trichotomous on the branches of the first order; further branching similar; terminal branchlets usually flask-shaped and distinctly pointed at the apex. Conidia always borne singly on the branchlets, soon falling away. round, elliptical, ovoid, inverted egg-shaped, or short spindle-shaped, hyaline or slightly colored.

KEY TO THE SPECIES OF THE GENUS VERTICILLIUM

- |                                   |                          |
|-----------------------------------|--------------------------|
| a. Colonies white.                | 1. <i>V. terrestre</i> . |
| aa. Colonies green.               | 2. <i>V. glaucum</i> .   |
| aaa. Colonies lightly olivaceous. | 3. <i>V. alboatrum</i> . |



Fig. 47. *Verticillium*. (After Lindau).

1. *Verticillium terrestre* (Link) Lindau (37)

Colonies pure white, spreading, floccose, consisting of dense, cobwebby, branched hyphae; conidiophores erect, septate, usually with four whorls of branchlets, branchlets rarely again verticillately branched; conidia formed singly at the tips of the branchlets, globose to elliptical, hyaline,  $4.4$  to  $5.0\mu$  by  $3.5$  to  $4.5\mu$ .

From soil: United States: Alaska (53), Iowa (3), Louisiana (2), New Jersey (52) (53)

\*2. *Verticillium glaucum* Bonorden (37)

Colonies spreading, blue green. Conidiophores erect,  $100$  by  $3\mu$ , twice verticillately branched, sparsely septate; branches usually trichotomously branched, secondary branches with three branchlets at the apex; conidia globose,  $2.5\mu$  in diameter, almost hyaline.

From soil: United States: New Jersey (52) (53)



\*3. *Verticillium alboatrum* Reinke and Berthold (37)

Mycelium spreading, brownish. Conidiophores erect, simple, dark colored, paler at the apex, with up to eight whorls, three to five branches in the whorl; branches sparsely septate, simple or further branched in whorls, terminal branchlets thickened at the base and narrowed at the apex, erect. Conidia elongate egg-shaped, hyaline, then brownish, 5 to 12 $\mu$  by 3 $\mu$ .

From soil: England (15)

Dale (15) reported the isolation of this species from soil in England. It is doubtful whether her species was *Verticillium alboatrum*, however, since she stated that her fungus was pure white in all stages. *Verticillium alboatrum* is lightly olivaceous.

Doubtful species

*Verticillium chlamydosporium* Goddard (20)

32. *Acrostalagmus* Corda 1838 (37)

Hyphae creeping, septate branched. Conidiophores erect, septate, usually branched in whorls. Conidia borne at the points of the branchlets, produced successively but not catenulate, forming a head held together by slime; conidia hyaline, egg-shaped to elliptical.

KEY TO THE SPECIES OF THE GENUS  
ACROSTALAGMUS

- a. Colonies floccose, pure white to creamy.
  - 1. *A. albus*.
- aa. Colonies not floccose, orange to avellaneous.
  - 2. *A. cinnabarinus*.

\*1. *Acrostalagmus albus* Preuss (37)

Colonies spreading, floccose, pure white or creamy. Sterile hyphae creeping, indistinctly septate, sparingly branched. Conidiophores arise as side branches of aerial hyphae, erect, up to 200 or 220 $\mu$  long, sometimes simple, but usually with one or two whorls of branchlets; branchlets non-septate, pointed, each bearing conidia on the point. Conidia hyaline, elliptical 3.0 to 3.5 by 1.0 to 1.5 $\mu$ .

From soil: United States: Alaska (53), Iowa (3) (33), Louisiana (2), New Jersey (52) (53), North Dakota (53), Texas (53)

\*1a. *Acrostalagmus albus* Preuss var. *varius* Jensen (26)

Colonies effused, thin, subfloccose, white; vegetative hyphae hyaline, branched, septate, 2 to 3.5 $\mu$ ; conidiophores creeping, ascending, or erect, branched, 15 to 75 $\mu$  by 2 to 3.5 $\mu$ ; usually simple but occasionally alternately branched, verticillate, alternate toward apex, slightly curved at the summit producing a head of conidia 15 to 36 by 2 to 3 $\mu$ , conidia hyaline, oblong, 3.3 by 1.5 $\mu$ .

From soil: United States: New York (26)



Fig. 48. *Acrostalagmus*. a—conidiophore; b—c conidial heads. (After Lindau).

\*2. *Acrostalagmus cinnabarinus* Corda var. *nana* Oudemans (26)

Colonies orbicular, orange mixed with red; vegetative hyphae septate; conidiophores septate, with two or three series of opposite branchlets, branches terminated by 3-rayed verticils, with each ray in the form of a ninepin, 36 to 45 $\mu$  long, bearing the conidia. Conidia elliptical or oblong 5 to 8 by 3 to 5 $\mu$ , formed in a head enveloped by slime.

From soil: Holland (32)

United States: Hawaii (52), Michigan (20), North Dakota (52) (53), New Jersey (52) (53), Oregon (52), Porto Rico (52) (53)

33. *Nematogonum* Desmazieres 1834 (37)

Hyphae creeping. Conidiophores erect with sterile and fertile cells. Sterile cells thickened on both sides. fertile cells globosely swollen, smooth. Conidia formed singly, ovoid, bright colored.

\*1. *Nematogonum humicola* Oudemans (37)

Turf circular, ribbon-like, at first white, then bright grey, finally cream colored. Conidiophores erect, 2.2-3.3 $\mu$  thick, hyaline, septate, unbranched, with longer cells not swollen at both ends and shorter smooth-walled fertile cells swollen at both ends. Conidia globose 3-4 $\mu$  in diam., or ellipsoid, 3-6 $\mu$  long and 2-4 $\mu$  broad, sessile, almost hyaline.

From soil: England (15), Holland (32)

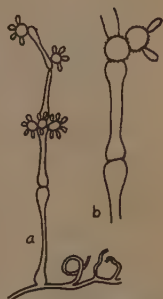


Fig. 49. *Nematogonum*. (After Lindau).

34. *Trichothecium* Link 1824 (17)

Hyphae creeping. Conidiophores erect, septate unbranched. Conidia terminal, single, 2-celled, hyaline or bright colored.

1. *Trichothecium roseum* Link

Syn: *Cephalothecium roseum* Corda

Turf forming a powdery case, widespread, mold-like or arachnoid, white, finally pink, formed of creeping, branched, septate white hyphae. Conidiophores erect, little or non-septate, usually unbranched and scarcely swollen at the tip. Conidia acrogenous, single, one after another, but remaining attached and forming a head by apical growth, pear-shaped, 2-celled, the apical cell being larger, hyaline then pink, 12-18 $\mu$  long by 8-10 $\mu$  broad.

From soil: England (15)

United States: Hawaii (52), North Dakota (52), New Jersey (53), New York (26), Porto Rico (53)



Fig. 50. *Trichothecium*. (After Lindau).

35. *Mycogone* Link 1824 (37)

Hyphae branched, interwoven. Conidiophores short, occurring laterally. Conidia single on the tips of the conidiophores, dissimilar, 2-celled, the upper cell larger, usually warty, bright colored, the lower cell pale. As in *Sepedonium* there are found here conidia and chlamydospores.

\*1. *Mycogone nigra* (Morgan) Jensen. (26)

Colonies at first hyaline, later showing yellowish tint, and finally becoming black-brown and zonate. In rapidly growing colonies, the hyphae near the margin are aerial as well as immersed and show a distinct yellow tint. Mycelium branched, septate, with numerous fertile branches bearing a single spore at the apex, 2.5-4 $\mu$  thick; conidiophores varying from scarcely none to a length of 30 $\mu$ , width 2-3.5 $\mu$ , ascending or erect; conidia uniseptate, upper cell dark brown, smooth, thick-walled, globose, 12-15 $\mu$  in diameter, lower cell hyaline to slightly colored, smooth, hemispherical, 8-10 by 9-12 $\mu$ . Intercalary cells are often formed. Variations in which the lower cell is not cut off, and again when a second small cell is formed, occur in culture. All conidia may probably be considered as chlamydospores.

From soil: United States: New York (26)

36. *Ramularia* Unger (18)

Conidiophores growing on sporodochia, simple or irregularly slightly branched, carrying the conidia on short teeth at their tips. Conidia egg-shaped, cylindric, finally 3 or more celled, seldom occurring in chains, hyaline or also bright colored. Parasites on living plants.

\*1. *Ramularia eudidyma* Wollenweber (42)

Conidia nearly cylindrical, with both ends rounded or irregularly slightly papillate, mostly 1-septate, 23 x 4.87 (21-26 x 4.7-5) $\mu$ , 0- to 2-septate conidia also found, 3-septate very rare; chlamydospores mostly intercalary, 8-11 $\mu$  in diam.; color of spore mass, from white to yellowish; color of plectenchyma, dense brown.

From soil: Europe (59)

\*2. *Ramularia macrospora* Fresenius (59)

Conidia scattered in sporodochia or as pionnotes, in mass buttery yellow to white. Scattered conidia ellipsoid, one-celled or septate, sporodochia or pionnotes conidia cylindrical, one to three septate. Dimensions: 1-celled, 7 to 20 by 3 to 5 $\mu$ ; 1-septate, 15 to 30 by 3.5 to 6.5 $\mu$ ; 2- and 3-septate, 25 to 40 by 4 to 7.5 $\mu$  (max. 50 $\mu$  long by 9 $\mu$  broad). Conidiophores seldom in sporodochia with side branches arranged in 2 to 3 membered whorls, usually simple or not branched. Plectenchyma and chlamydospores chest-



Fig. 51. *Mycogone*.  
(After Lindau).



Fig. 52. *Ramularia*.  
(After Lindau).

nut brown, especially on starchy media, brown, round, thick-walled chlamydospores in chains or knots, more frequently than single; terminal or intercalary, 10 to 16 $\mu$  broad.

From soil: Europe (59)

*Ramularia magnusiana* (see *Neonectria ramulariae*)

## XII. DEMATIACEAE

### 37. *Stachybotrys* Corda 1837 (37)

Mycelium creeping, spreading over the substratum, septate, branched, hyaline, or slightly colored. Conidiophores arise as branches of the mycelium, erect, variously branched, septate, dark colored or almost hyaline, bearing at the apex of the main stalk and branches small sterigma-like cells, which are non-septate, hyaline or slightly dark colored, and either borne in whorls or arise irregularly below the point of the branch, appearing single or more or less grouped. Conidia borne singly on the points of the sterigmata round or elongate, black, smooth or echinulate.

#### KEY TO THE SPECIES OF THE GENUS STACHYBOTRYS

- a .Branching of conidiophores regularly alternate.
  - 1. *S. alternans*.
- aa. Branching of conidiophores not regularly alternate.
  - b. Conidiophores short, up to 75 $\mu$  long, conidia smooth.
    - 2. *S. atra*.  
(*S. cylindrospora*).
  - bb. Conidiophores up to 1 mm. long, conidia echinulate.
    - 3. *S. lobulata*.



Fig. 53. *Stachybotrys*. (After Lindau).

### \*1. *Stachybotrys alternans* Bonord. (37)

Sterile hyphae creeping, branched, sparsely septate, black-brown, 3 to 5 $\mu$  thick, with abundant papillae. Conidiophores erect, gray or almost hyaline, 3.5 $\mu$  thick, mostly unbranched, branching when present regularly alternate, not swollen at the apex, with crowded, inverted egg-shaped or club-shaped sterigmata, gray or hyaline, 10 $\mu$  by 4 to 5 $\mu$ . Conidia borne on the ends of the sterigmata, elliptical to ovoid, with or without two oil drops, black, roughened, 8 to 12 $\mu$  by 5 to 7.5 $\mu$ .

From soil: United States: Porto Rico (53)

### \*2. *Stachybotrys atra* Corda (26)

Colonies spreading, at first hyaline, becoming black with age; mycelium hyaline, septate, 5 to 6 $\mu$  thick, with branches almost at right angles, and with oval, ellipsoidal or globose chlamydospores up to 12 $\mu$  in diameter; articulate with age. Conidiophores arise from aerial mycelium, fuliginous near the apex, almost hyaline near the base, branched, septate, 65 to 74 $\mu$

long by 2 to 4 $\mu$  thick, slightly alternate toward the apex, bearing on the summit a whorl of papillate sterigmata; sterigmata 10 to 12 $\mu$  by 4.5 to 5 $\mu$ . Conidia single, smooth, elliptical, usually with acute ends and mostly with two oil drops, slightly colored when young to fuliginous and black when mature.

From soil: United States: New York (26)

2a. *Stachybotrys cylindrospora* Jensen (26)

Jensen's description of *S. cylindrospora* so closely resembles that of *S. atra*, it seems probable that they should be considered synonymous.

Colonies round, thin, diffuse, becoming black with age; mycelium branched, septate, hyaline, 0.5 to 3 $\mu$  thick. Conidiophores hyaline at base, fuliginous toward apex, branched, septate, attenuate toward tip, 40 to 65 $\mu$  high, bearing on the summit from three to nine sterigmata; sterigmata sub-clavate, with or without short papillae, 8 to 11 $\mu$  by 4 to 5 $\mu$ ; conidia borne singly, smooth, subcylindrical to sometimes ovate, 6 to 16 $\mu$  by 3.8 to 5 $\mu$ , hyaline when young, becoming fuliginous with age.

From soil: United States: Iowa (33), New York (26)

3. *Stachybotrys lobulata* Berkeley (37)

Colonies broadly spreading, black, dense; hyphae creeping, almost hyaline, septate. Conidiophores arise from aerial mycelium, erect, up to about 1 mm. long by 3 to 4 $\mu$  thick, septate, almost hyaline at the base, darker toward the apex, with few branches, 30 to 35 $\mu$  long, which are granular within. Sterigmata borne at the apex of the branches, usually three to five, black, finely warty, 11 to 12 $\mu$  by 6 $\mu$ , each bearing a conidium. Conidia black, finely warty or echinulate, round to elliptical, 9 to 12 $\mu$  by 7 to 8 $\mu$ .

From soil: Iowa (3)

38. *Gliobotrys* von Höhnelt 1902 (37)

Sterile hyphae sparse, creeping. Conidiophores hyaline, erect, scarcely swollen at the tip, carrying a thick crown of short hyaline, simple branchlets placed in a whorl. Conidia olive-green, ellipsoid, encased in slime and forming a round head.

1. *Gliobotrys alboviridis* v. Höhnelt (37)

Conidiophores hyaline with 1-5 cross-walls, usually unbranched, 120 $\mu$  long, 5-8 $\mu$  thick, a little thicker at the tip, carrying at the end 5-8 simple non-septate, cylindric branches, 10-12 $\mu$  long. Conidia egg-shaped, bright olive or green, 4-6 $\mu$  long, 3-4.5 $\mu$  thick, encased in slime and forming a spherical head.

From soil: United States: Iowa (33)

39. *Periconia* (Tode 1691) Bonorden 1851 (37)

Sterile hyphae creeping, abundant, scarcely transparent. Conidiophores erect or reclining, unbranched, brown, more or less swollen at the apex where the conidia are borne, seldom with short branchlets at the apex. Conidia borne singly, globose or ovoid, brown.



Fig. 54.  
*Periconia*.  
(After  
Lindsau).



## KEY TO THE SPECIES OF THE GENUS PERICONIA

- a. Colony brown; conidia finely echinulate. *P. lanata*.  
 aa. Colony white; conidia smooth. *P. byssoides*.

\*1. *Periconia byssoides* Pers. (37)

Conidiophores grouped to form a colony, thread-like, rather rigid, septate, brown, light colored at the apex, about 1 mm. in height. Conidial heads globose, firm. Conidia globose, rather large, finely echinulate, dark brown, 5 to 7 $\mu$  in diameter.

From soil: United States: Idaho (36)

2. *Periconia lanata* n. sp.

Colonies spreading, floccose woolly white on the surface, reverse at first white, later yellow to brown, spotted by occurrence of dark brown to black sclerotia. Conidiophores prostrate or ascending, very variable in length up to 100 $\mu$ , hyaline; conidia borne at the apex in an irregular head, on short sterigmata with inflated bases, globose to ovoid 3 x 9 $\mu$ , usually 5-7 $\mu$  in diam., smooth, dark brown.

From soil: Louisiana

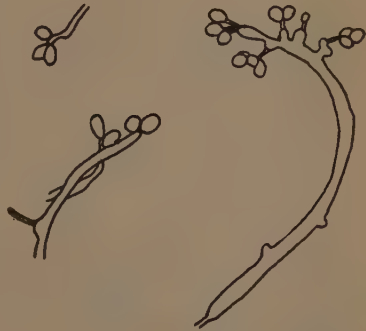


Fig. 55. *Periconia lanata*, n. sp.

40. *Synsporium* Preuss 1851 (37)

Sterile hyphae creeping. Conidiophores forming a turf, erect, septate, branched. Conidia oblong, borne in heads at the apex of the conidiophores, brown non-septate. (The genus is *Acrothea* with branched conidiophores).

\*1. *Synsporium biguttatum* Preuss (37)

Colonies spreading, at first dark, then coal black. Conidiophores creeping, then erect, branched, brown. Conidia large, ovoid, at first hyaline, then black brown, usually with an oil drop at each end

From soil: England (16)



Fig. 56. *Synsporium*.  
(After Lindau).

41. *Basisporium* Molliard 1902 (29)

Hyphae creeping, at first hyaline, later dark, ultimate branchlets bearing cushion-like basidia either laterally or terminally. Conidia solitary, sub-globose, smooth. This genus approaches the genus *Pachybasium* among the Botrytideae and Rhinocladium among the Trichosporieae.

1. *Basisporium gallarum* Molliard (29)

Both sterile and fertile hyphae creeping, at first hyaline, then dark; sterile hyphae septate 18 $\mu$  in diameter; fertile hyphae septate, 4 $\mu$  in diam., much branched bear-



Fig. 57. *Basisporium*.  
(After Lindau).

ing swollen jar-like cells terminally and laterally on which are borne singly the suspherical, smooth, black conidia. Conidia one-celled, 11-14 $\mu$  in diam.

From soil: England (15)

United States: New Jersey (53)

42. *Acremoniella* Saccardo 1886 (37)

Hyphae creeping or slightly ascending, unbranched or branched, hyaline or dark colored, bearing here and there short side branches, which bear conidia on the points. Conidia round or ovoid, borne singly, brown, one-celled.

KEY TO THE SPECIES OF THE GENUS *ACREMONIELLA*

a. Conidia globose, smooth.

1. *A. fusca* var. *minor*.

aa. Conidia pyriform, verrucose.

2. *A. brevia*.



Fig. 58. *Acremoniella*. (After Lindau).

1. *Acremoniella fusca* Kunze var. *minor* Corda (52)

Colonies spreading, greenish brown. Hyphae yellowish-brown, forked, with irregularly placed side branches. Conidia round, pale ochre color, transparent, finally olive green.

From soil: United States: Alaska (53), Iowa, North Dakota (53)

2. *Acremoniella brevia* n. sp.

Colonies on Czapek's agar spreading, felty or closely floccose; surface dark grayish to brownish green, with whitish superficial hyphae appearing in the center of old colonies. Reverse green black to black. Colonies consist of abundant, dark colored verrucose, multiseptate aerial mycelium, bearing very short scattered conidiophores, 2 to 15 $\mu$  long, verrucose, dark colored. Conidia borne terminally, pyriform to sub-globose, delicately rugulose, dark brown, 6.5 to 9.5 $\mu$  by 5 to 6 $\mu$ , one-celled; conidia sometimes almost sessile. Chlamydospores common.

From soil: United States: Louisiana



Fig. 59. *Acremoniella brevia*, n. sp.



Fig. 60. *Dematium*. (After Lindau).

43. *Dematium* Persoon 1797 (37)

Hyphae creeping, little developed or forming a turf. Conidiophores erect, unbranched or sparsely branched, septate, carrying conidial chains laterally. Conidia globose, or ovoid, sometimes held together by short interstitial cells, one-celled, dark colored.

1. *Dematium scabridum* n. sp.

Grows poorly on Czapek's agar. On bean agar, abundant growth of floccose, aerial mycelium, olive brown or dark olivaceous in color, becoming almost black; reverse uncolored. Conidiophores arise from submerged or surface hyphae, often once forked, septate, up to  $50\mu$  long. Sterile hyphae multi-septate, cells often swollen, olivaceous. Conidia borne terminally on the conidiophores in very long chains which spread over the medium. Mature conidia pear-shaped, brown at first but becoming black when mature, thick walled, markedly verrucose,  $15$  to  $23\mu$  by  $13$  to  $18\mu$ .

From soil: United States: Louisiana

Fig. 61. *Dematium scabridum*, n. sp.44. *Hormodendrum* Bonorden 1851 (37)

Sterile hyphae creeping, branched, septate. Conidiophores erect, septate, brown, variously branched or only little branched. Conidial chains acrogenous on the branches (often all the branches are borne on a single main stipe). Conidia globose or ovoid, olive green or brown, one-celled.

KEY TO THE SPECIES OF THE GENUS  
*HORMODENDRUM*

- a. Colonies olive green.
  - b. Conidia  $3-6 \times 2.5-3.6\mu$ .
  - bb. Conidia  $8-12 \times 4-5\mu$ .
  - bbb. Conidia  $4-12 \times 2-4\mu$ .
- aa'. Colonies not olive green.
  - b. Colonies brown.
  - bb. Colonies gray.
  - bbb. Colonies black.

Fig. 62. *Hormodendrum*. (After Lindau).

- 1. *H. cladosporioides*.
- 2. *H. olivaceum*.
- 3. *H. viride*.
- 3. *H. hordei*.
- 4. *H. pallidum*.
- 5. *H. nigrescens*.

1. *Hormodendrum cladosporioides* (Fresenius) Saccardo (26)

Syn: *Cladosporium herbarum* (Persoon) Link

Colonies dark olivaceous green, round, dense; conidiophores erect, branched,  $100$  to  $200\mu$  long, olivaceous, toward the apex gradually attenuate,

ultimate branches copiously dividing with predominant tendency to dichotomy, septate, articulate above; conidia cylindrical to broadly oval, olivaceous, smooth, 3 to 6 $\mu$  by 2.5 to 3.6 $\mu$ , continuous or inferior ones rarely septate.

From soil: England (15) (16)

United States: California (53), Iowa (3) (33), Louisiana (53), Michigan (19) (20), New Jersey (53), New York (26), Oregon (52) (53), Texas (53)

2. *Hormodendrum olivaceum* (Corda) Bonorden (33) (37)

Colonies olive green, spreading. Conidiophores erect, unbranched except at the apex, olive green, 75 to 200 $\mu$  long, born as lateral branches of the sterile hyphae. Conidial chains short. Conidia elliptical to short cylindrical, 8 to 12 $\mu$  by 4 to 5 $\mu$ , with intermediate cells of the chain swollen, and terminal cells often much smaller and globose.

From soil: Iowa (33)

3. *Hormodendrum viride* (Fresenius) Saccardo (37) (33)

Colonies gray-green, small. Conidiophores arising from prostrate mycelium, erect, septate, branched at the tip and ending in forked conidial chains. Conidia long or egg shaped, frequently with 2 oil-drops, green, smooth, 4-12 $\mu$  x 2-4 $\mu$  (Lindau (37) gives the conidial measurements as 7-8 $\mu$  long).

From soil: United States: Iowa (33)

\*4. *Hormodendrum hordei* Bruhne (26)

Colonies brown at maturity, circular, dense; mycelium brown, septate, branched, 3 to 6 $\mu$  thick; conidiophores simple septate, ascending or erect, 50 to 100 $\mu$ ; conidia various, some cylindrical with ends rounded, truncate, or subattenuate, others ellipsoidal, ovate, or subglobose, regular or somewhat angular; with age many become once septate and verrucose, 4 to 14 $\mu$  by 3 to 5 $\mu$ , chains of conidia short.

From soil: United States: Alaska (53), New York (26)

\*5. *Hormodendrum pallidum* Oudemans (26)

Colonies orbicular, gray, not plainly zonate. Conidiophores erect, very light gray, upward dendroidly branched; primary and even secondary branches decussate, each succeeding branch and branchlet shorter than preceding, consisting of single cells, constricted at septa. Conidia variable in size, 12 to 20 $\mu$  by 5 to 8 $\mu$ .

From soil: Holland (32)

5. *Hormodendrum nigrescens* Paine (33)

Colonies somewhat elevated, at first hyaline, becoming olive green, and finally black beneath with white surface; the hyaline mycelium appears slightly floccose; margin 2 mm. or more wide, hyaline. Sterile hyphae arise at the apex of the colony as fine bristle-like tufts above the conidiophores.

Conidiophores originating in the substratum, smoky, 300 to 400 $\mu$  long by 4.5 to 5 $\mu$  in thickness, dendroidally branched, erect. Conidia green, sub-spherical to ellipsoidal or spindle-shaped, seldom pointed at the ends, 4 to 10 $\mu$  by 2.5 to 4 $\mu$ .

From soil: United States: Iowa (33)

45. *Mesobotrya* Saccardo (37)

Conidiophores erect, dark colored, with whorls of fertile branchlets arising in the middle portion; apex sterile. Conidia borne terminally on the branchlets; ovoid, hyaline.

1. *Mesobotrya simplex* n. sp.

Colonies on Czapek's agar dark olive to brown green, slowly spreading, velvety to sub-floccose, consisting of both submerged and aerial hyphae; reverse greenish black. Conidiophores arise in groups from aerial hyphae, with aerial and submerged stolons surrounding the point of origin, swollen at the base, and tapering to a pointed apex; brown in color; sparsely and indistinctly septate; bearing on the middle portion whorls of fertile branchlets, which may also be once branched. Conidiophores 150 to 350 $\mu$  long by 5 to 6 $\mu$  in diameter near the base; branches up to 100 $\mu$  long. Conidia borne singly and terminally on the branches, oval to ovoid, light brown green, smooth, 3.5 to 5 $\mu$  by 3 to 3.5 $\mu$ .

From soil: United States: Louisiana

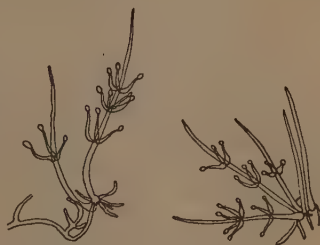


Fig. 63. *Mesobotrya simplex*, n. sp.

46. *Dicoccum* Corda 1829 (37)

Hyphae creeping, branched, septate, dark colored. Conidia terminal on short, erect side branches, elongate or short clavate, two-celled, sometimes biscuit form, dark colored.

\*1. *Dicoccum asperum* Corda (52)

Syn: *Trichocladium asperum* Harz

Colony floccose, white at first, becoming dark to almost black with a shade of yellow; reverse yellowish black. Mycelium consists of hyaline, branched, sparsely septate, yellowish hyphae, 2.6 to 3.5 $\mu$  thick; conidiophores arise as short side branches, 3 to 12 $\mu$  long. Conidia at first hyaline, then brown to black, oval, two-celled; upper cell spherical, brown, thick-walled, spiny; lower cell usually smaller, spiny; conidia 20 to 22 $\mu$  by 9 to 13 $\mu$ .

From soil: United States: Colorado (53), New Jersey (52)



Fig. 64. *Dicoccum*.  
(After Lindau).



47. *Cladosporium* Link 1816 (37)

Hyphae creeping, septate, on the surface or in the substrate. Conidiophores almost erect, branched, and floccose, often forming a turf, olive colored. Conidia globose and ovoid, at first one-celled, then usually with a cross-wall, usually greenish, terminal and then pressed to the side.

\*1. *Cladosporium epiphyllum* Persoon (37)

Colonies greenish black, large, thick; conidiophores at first erect, then falling, pale green; conidia very numerous, soon falling from the chain, at first one-celled, then 2 to more celled, olive green,  $10-22\mu$  long  $\times$   $4-6\mu$  thick.

Waksman (52) gives: conidia 1- or 2-celled,  $10-14\mu \times 3.8-5.2\mu$ .

From soil: England (15) (16)

United States: California (52), New Jersey (52), Texas (53)

48. *Scolecobasidium* Abbott (4)

Hyphae creeping, septate; conidiophores arising as short side branches from aerial hyphae, not erect, non-septate. Conidia elongate, two-celled, smooth, light olivaceous to almost hyaline, borne singly on short, terminal, thread-like sterigmata; one to three sterigmata on each conidiophore.



Fig. 65. *Scolecobasidium*.

1. *Scolecobasidium terreum* Abbott (4)

Cultivated on dextrose bean agar, colonies round, 2-3 cm. in diameter; surface velvety, olivaceous; reverse greenish black. Hyphae light olivaceous, septate. Conidiophores  $5.0$  to  $8.0\mu$  long by  $2.0$  to  $2.5\mu$  wide. Sterigmata  $0.5$  to  $1.0\mu$  long. Conidia T- or Y-shaped, two celled, light olivaceous to almost hyaline, smooth,  $4.0$  to  $12.0\mu$  long by  $2.0$  to  $2.5\mu$  wide. Perithecia or sclerotia not observed.

From soil: United States: Louisiana (4)

2. *Scolecobasidium constrictum* Abbott (4)

Cultivated on dextrose bean agar, colonies round, seldom more than 3 cm. in diameter; surface fuscous, olivaceous; reverse greenish black. Hyphae light olivaceous, septate. Conidiophores  $5.0$  to  $8.0\mu$  long by  $2.0$  to  $2.5\mu$  wide. Sterigmata  $0.5$  to  $1.0\mu$  long. Conidia two-celled, slightly constricted at the center, smooth, light olivaceous,  $6.0$  to  $12.0\mu$  long by  $2.5$  to  $4.0\mu$  wide. Perithecia or sclerotia not observed.

From soil: United States: Louisiana (4)

49. *Helminthosporium* Link 1809 (37)

Colonies consist of conidiophores, loose or dense, regularly or irregularly velvety, brown to black, with strict or spreading margin. Conidiophores usually arise in groups, erect and straight, sometimes reclining, usually unbranched, only seldom with small side

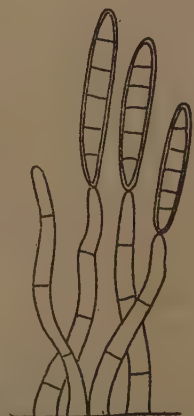


Fig. 66. *Helminthosporium*. (After Lindau).

branches, septate, geniculate at points below the conidia, brown, green-brown to black, transparent or non-transparent. Conidia terminal or lateral on the geniculations, elongate, cylindrical, clavate or obelavate, smooth, mostly rounded at both ends, or sometimes pointed at the base or at both ends, straight or bent, with more than one cross wall, dark brown, green brown to black, often with the end cells lighter colored.

#### KEY TO THE SPECIES OF THE GENUS *HELMINTHOSPORIUM*

- a. Colonies floccose, conidia 22 to 26 by 9 to 11 $\mu$ .
  - 1. *H. subulatum*.
- aa. Colonies velvety, conidia 15 to 23 by 4.5 to 7.5 $\mu$ .
  - 2. *H. interseminatum*.
- aaa. Colonies velvety, conidia 40 to 90 by 10 to 15 $\mu$ .
  - 3. *H. anomalum*.

#### \*1. *Helminthosporium subulatum* Nees (37)

Colonies floccose. Conidiophores usually unbranched, straight, 11 $\mu$  thick at base, 6 to 7 $\mu$  thick at the apex. Conidia cylindric-ellipsoid, rounded at the apex, often attenuated at the base, with 3 to 4 septa, black brown, 22 to 26 $\mu$  by 9 to 11 $\mu$ .

From soil: Japan (45)

#### \*2. *Helminthosporium interseminatum* Berk. et Rav. (37)

Colonies broadly spreading, brown black, velvety. Conidiophores grouped thickly together, erect, also sometimes reclining, unbranched or branched, multiseptate, bent or with geniculations, brown, transparent, sometimes swollen and with knobs at the apex, up to 500 $\mu$  long, by 3.5 to 4 $\mu$  thick. Conidia borne terminally or laterally on the geniculations, slender elongate, rounded at both ends, almost always with three septa, more seldom with two or four, cells of the same size, brown, transparent, 15.5 to 23 $\mu$  by 5.5 to 7.5 $\mu$ .

From soil: England (15)

#### 3. *Helminthosporium anomalum*, n. sp.

Colonies on Czapek's agar slowly but broadly spreading, at first consisting largely of submerged hyphae, but later developing aerial hyphae and conidiophores; velvety; surface greenish black to black, reverse black; aerial mycelium dark brown, submerged mycelium dark brown to almost black, multiseptate. On bean agar colonies become floccose and are dark brown green in color. Conidiophores arise usually from submerged hyphae, more or less bent, and bearing a terminal group of conidia, with lateral conidia borne singly and irregularly on the geniculations; conidiophores mostly 150 to 400 $\mu$  long, brown; conidia elongate, straight, rounded at both ends, 5 to 12 times septate, mostly 7, when mature 40 to 90 $\mu$  long by 10 to 15 $\mu$  broad.

From soil: United States: Iowa, Utah



Fig. 67. *Helminthosporium anomalum*, n. sp.

50. *Spondylocladium* Martius 1817 (37)

Hyphae creeping, septate. Conidiophores erect, unbranched, slightly rigid. Conidia borne in lateral whorls, spindle-shaped, usually 3-celled, dark colored.

1. *Spondylocladium australe* n. sp. Sm

Colonies on Czapek's agar spreading, floccose, aerial hyphae abundant; surface dark grayish to olivaceous green, with an olive gray floccose overgrowth in old cultures; reverse greenish black to black. Conidiophores arise from aerial mycelium, erect, multiseptate, geniculate, dark colored, bearing conidia terminally and laterally, either singly or in groups of 2 to 6. Apex of conidiophore often slightly swollen. Conidiophores 80 to 250 $\mu$  long. Conidia borne in a terminal whorl and laterally on the geniculations, 25 to 38 $\mu$  by 12 to 15 $\mu$ , often slightly curved, smooth, three septate. The two central cells are about twice as large as the end cells, and are dark colored, while the end cells are nearly hyaline.

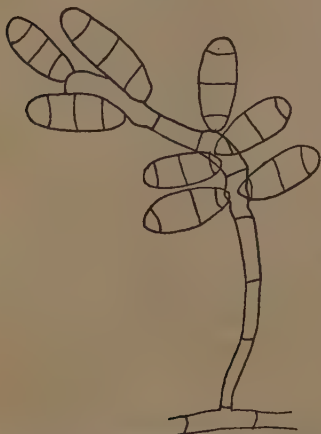


Fig. 69. *Spondylocladium australe*, n. sp.



Fig. 68. *Spondylocladium*. (After Lindau).

From soil: United States: Louisiana

2. *Spondylocladium xylogenum*  
A. L. Smith (43)

Colonies on Czapek's agar spreading, velvety, surface dark gray green to black green or black. Reverse black. Conidiophores arise from aerial mycelium, erect, dark colored, septate, geniculate, 75 to 150 $\mu$  long. Conidia borne terminally and laterally on the conidiophores, very thickly on the stalks, 15 to 25 $\mu$  by 8.0 to 13.5 $\mu$ , three septate, curved slightly.

From soil: United States: Louisiana

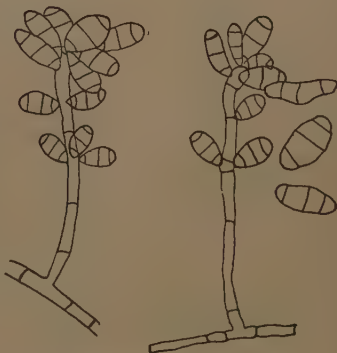


Fig. 70. *Spondylocladium xylogenum*.

51. *Acrothecium* Preuss 1851 (37)

Hyphae creeping, slightly raised. Conidiophores erect, undivided, dark colored. Conidia long or spindle-shaped, 3- or more celled, colored or almost hyaline forming a terminal head.

1. *Acrothecium robustum* n. sp.

Colonies on Czapek's agar broadly spreading, velvety, consisting mostly of submerged mycelium and aerial conidiophores, with little aerial mycelium; surface black, reverse black. Conidiophores arise from submerged or aerial hyphae, multi-septate, dark colored, thick-walled, smooth, 50 to 150 $\mu$  long, averaging about 100 $\mu$ . Conidia borne typically in terminal heads, but are occasionally produced laterally on the conidiophores; apex of the conidiophores very slightly inflated. Conidia elongate, barrel-shaped, 4- or 5-septate, thick-walled, dark colored, smooth, 37 to 50 $\mu$  by 10 to 14 $\mu$ .

From soil: United States: Louisiana, Utah



Fig. 71. *Acrothecium*. (After Lindau).

52. *Tetracoccusporium* Szabo 1905 (37)

Conidiophores septate, branched, hyaline smoky. Conidia globose at the tips of short branches, black brown, divided by two partitions at right angles to each other into four cells.

1. *Tetracoccusporium paxianum* Szabo

Syn: *Stemphylium paxianum*

Colonies on bean agar broadly spreading, margins of the colony finger-like; surface velvety, gray to greenish black or black; reverse uncolored. Aerial mycelium multiseptate, olivaceous, 4 to 6 $\mu$  thick. Conidiophores arise from submerged or aerial mycelium, sometimes once branched, multiseptate, olivaceous, smooth, up to 75 $\mu$  by 4 to 5 $\mu$ . Conidia borne terminally and laterally, singly or in heads of 3, 4, or 5; 4-celled, cruciately septate, black, markedly verrucose. In old cultures conidial walls are so thick the septa are seen with difficulty. Conidia pear-shaped, 17 to 25 by 12 to 17 $\mu$ .

From soil: United States: Idaho (36), Utah



Fig. 72. *Acrothecium robustum*, n. sp.



Fig. 73. *Tetracoccusporium*. (After Lindau).

53. *Stemphylium* Wallroth 1833 (37)

Sterile hyphae, creeping, spreading, mostly dark colored, septate, floccose. Conidiophores arise as side branches, more or less erect, often very short, mostly unbranched and often non-septate. Conidia borne singly and terminally, ovoid or almost club-shaped, often a little pointed, muriform, more or less dark colored to non-transparent.

KEY TO THE SPECIES OF THE GENUS *STEMPHYLIUM*

- a. Colonies black, conidia smooth.
  - b. Pear-shaped or oval, constricted at apex.
    - 1. *S. piriforme*.
  - bb. Elliptical to almost globose, not constricted at apex.
    - 2. *S. botryosum*.
- aa. Colonies dark olive, conidia verrucose.
  - 3. *S. verruculosum*.



Fig. 74. *Stemphylium*. (After Lindau).

\*1. *Stemphylium piriforme* Bonorden (37)

Colonies somewhat spreading, black. Hyphae freely branched, creeping, septate, smoky. Conidia terminal on the conidiophores, inverted pear-shaped or oval, muriform with three to four cross-walls, slightly constricted at the septa, black gray, 25 to 30 $\mu$  by 12 to 15 $\mu$ , smooth.

From soil: United States: Idaho (36)

\*2. *Stemphylium botryosum* Wallroth (37)

Colonies very dark, orbicular; vegetative hyphae creeping, spreading, thin, irregularly branched, at first hyaline, then becoming light brown and finally dark brown, septate, flexuose, more or less moniliform. Conidiophores arise as branches, short, flexuose, simple or branched, hyaline or colored, more or less rough, simple or forked at the summit. Conidia terminating the primary as well as the secondary branches, with short pedicel, sometimes nearly globose, sometimes elliptical or oblong, divided horizontally into 2 to 6 compartments, of which one or several present a vertical or oblique septum, isabel-colored to brownish-black, 25 to 40 $\mu$  by 16 to 20 $\mu$ ; surface of conidia with age finely dotted.

From soil: England (16)

\*3. *Stemphylium verruculosum* Zimmermann (37)

Colonies spreading, dark olive green. Hyphae curved, hyaline, branched, about 22 $\mu$  thick, with short or long branches. Conidia inverted, egg-shaped or elliptical, with two or three septa, muriform, verrucose, brown, non-transparent when mature, 17.5 to 22 $\mu$  by 11 to 13.5 $\mu$ .

From soil: Japan (45)



54. *Macrosporium* Fries 1832 (37)

Sterile hyphae dark brown to almost black; conidiophores seldom single, mostly arising in groups, erect, flexuous or almost straight, septate, often with the upper cells somewhat swollen, usually unbranched, brown to black, usually transparent, conidia formed acrogenously, then being pushed laterally. Conidia terminal and single, ovoid or elongate, usually more or less club-shaped, sometimes drawn out to a light (hyaline) point, muriform, brown to black, often finely echinulate.

KEY TO THE SPECIES OF THE GENUS  
*MACROSPORIUM*

- a. Colonies greenish brown, conidiophores 150 to 200 $\mu$  long.  
1. *M. cladosporioides*.
- aa. Colonies black-brown, conidiophores up to 90 $\mu$  long.  
2. *M. commune*.



Fig. 75. *Macrosporium*.  
(After Lindau).

1. *Macrosporium cladosporioides* Desm. (37)

Colonies small, round, velvety, dark greenish brown. Conidiophores arise in groups, erect, unbranched, gnarled, septate, almost hyaline, 150 to 200 $\mu$  long, 5 $\mu$  thick. Conidia ovoid, elongate, or club-shaped, almost transparent, (sometimes torulate), muriform, with two to ten cross-walls, 15 to 75 $\mu$  long and 8 to 14 $\mu$  thick; ovoid conidia finely granular, club-shaped ones smooth.

From soil: England (15) (16)

2. *Macrosporium commune* Rabenhorst (37)

Colonies dense, brown to black-brown. Conidiophores arise in groups, ascending, usually unbranched, septate, not constricted at the septa, 80 to 90 $\mu$  long by 4 to 7 $\mu$  thick. Conidia very variable, inverted, egg-shaped, elongate, or club-shaped, narrowed at the base, with three to five cross-walls and several oblique transverse walls, olive green or olive brown, usually with finely granular surface, 18 to 35 $\mu$  by 8 to 14 $\mu$ .

From soil: United States: Idaho (36)

55. *Alternaria* Nees 1817 (37)

Sterile hyphae creeping, septate. Conidiophores single or in groups, erect, septate, mostly unbranched, short. Conidia inverted, club-shaped, mostly elongate at the tip, muriform in the lower portion, dark colored, lighter at the points, borne in more or less long, usually simple chains.

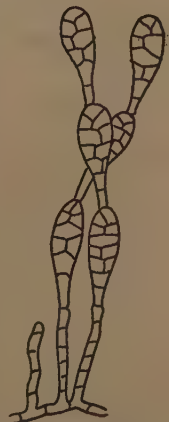


Fig. 76. *Alternaria*.  
(After Lindau).

## KEY TO THE SPECIES OF THE GENUS ALTERNARIA

a. Colonies black-green, conidia 50 by  $16\mu$ , rough.

1. *A. humicola*.

aa. Colonies brown, conidia 35-90 by 9 to  $14\mu$ , smooth.

2. *A. fasciculata*.

aaa. Colonies brown-green, conidia 30 to 36 by 14 to  $15\mu$ , smooth.

3. *A. tenuis*.

1. *Alternaria humicola* Oudemans (26)

Colonies at maturity orbicular, black-green; fertile hyphae well developed, hyaline, articulate, 3 to  $5\mu$  in diameter, racemously branched; conidia variable in shape, cylindrical, obelavate, oblong, lageniform, at first hyaline, later honey-colored, thin, dark, finally black-green and smoky, variable in size, maximum 16 by  $50\mu$ , 3 to 7 times septate, muriform, in advanced age dense and very finely roughened, slightly or non-constricted at the septa.

From soil: England (15), Holland (32)

United States: Iowa (3), Louisiana (2), New Jersey (52) (53), Utah

\*2. *Alternaria fasciculata* Cooke and Ellis (26)

Conidiophores brown, erect or ascending, irregularly curved, solitary or caespitose, septate, diameter uniform, 40 to  $130\mu$  by  $3\mu$ ; conidia dark brown, oblong ovate, minutely apiculate, 35 to  $90\mu$  by 9 to  $14\mu$ , endochrome transversely two to seven times septate with usually several longitudinal septa, the apical cell short or elongated into a straight hyaline beak.

From soil: United States: New York (26)

\*3. *Alternaria tenuis* Nees. (37)

Conidiophores short, septate, unbranched or branched, brown green. Conidia in chains, muriform with three to five cross-walls, constricted at the outer walls, olive green or brownish black, very variable in size and shape, 30 to  $36\mu$  by 14 to  $15\mu$ .

From soil: England (16), Japan (45)

XIII. STILBACEAE

56. *Stysanus* Corda 1837 (18)

Coremia erect, clubbed-cylindric, dark colored, rigid. Conidia occurring in a loose, long or almost globose panicle, ovoid or lemon shaped, almost hyaline formed in chains.

\*1. *Stysanus stemonites* (Pers.) Corda (37)

Coremia gregarious. Stalk thin, unbranched, brownish black, formed by a fascicle of elongated, septate, green-brown hyphae, ending at the tip in a cylindric head. Conidia ovate to lemon-shaped, bluish-green, transparent, formed in chains, 6-8 $\mu$  long by 4-5 $\mu$  in diam.

From soil: United States: Maine (53), Michigan (20), Texas (53)



Fig. 77.  
*Stysanus*.  
Lindau.  
(After

57. *Tilachlidium* Preuss 1851 (37)

Coremia formed of fasciculated thread-like hyphae, branched, the secondary branches being sterigma-like, awl-shape, somewhat club-shape at the tip, furnished with little heads, usually consisting of a single hypha carrying on its end a conidial head. Heads slimy, later dry. Conidia one-celled, ovate, hyaline, occurring at the end of the conidiophore.

\*1. *Tilachlidium humicola* Oudemans (37)

Coremia circular, snow-white; turf wooly. Main stalk upright, cylindric, 35-40 $\mu$  thick, made up of very delicate, articulate, closely interwoven hyphae, from all sides of which spring single hyphae as secondary branches, basidia-like, 40-80 $\mu$  long, erect unbranched, non-septate, curved, with an almost club-like head. Conidia bound together by mucus into a spherical, terminal, finally dry head, 15-18 $\mu$  in diameter, elongate or ovate, very bright green, 6-7 $\mu$  long, 3-5 $\mu$  thick.

From soil: Holland (32)

## XIV. TUBERCULARIACEAE

58. *Hymenula* Fries (18)

Conidial layer shield-shaped, regular, smooth bright colored. Conidiophores simple, seldom branched. Conidia egg-shaped, terminal single.

\*1. *Hymenula affinis* (Fautrey and Lambotte) Wollenweber (42)

Syn: *Fusarium affine* Fautr. and Lamb.

Conidia straight, somewhat dorsiventral near apex, apedicillate, typically 1-septate, 10.2  $\times$  2.8 (9-11.4  $\times$  2.6-3) $\mu$  usually in a continuous smooth or slight roughened, slimy-layer, from hyaline to pale salmon-colored on a glucose agar; conidiophores from simple to sparingly branched, septate; mycelium hyaline; no chlamydospores.

| From soil: United States: Idaho (36)

59. *Volutella* Tode 1790 (37)

Fruit layer superficial, disc-shaped or somewhat globose, sessile or on a short stalk, regularly formed, with long bristles or spines at the margin and sometimes in the middle of the disc. Conidiophores thickly gregarious, covering the entire disc, at the base united with the spines as branches, usually several times branched, the last branches forming a thick hymenium of fine,



Fig. 78. *Tilachlidium*. (After Lindau).



Fig. 79. *Hymenula*. (After Sherbakoff).

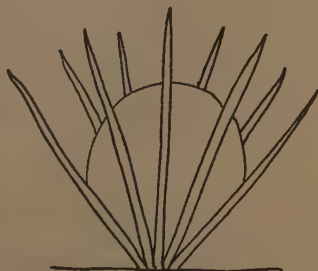


Fig. 80. *Volutella*. (After Lindau).

sterigma-like stalks. Conidia terminal, formed in masses, small, ovoid or elliptical, hyaline.

1. *Volutella piriformis* n. sp.

Colonies on Czapek's agar broadly spreading, brown or grayish brown with superficial whitish aerial mycelium in old cultures; reverse brownish black. Mycelium largely submerged, dark brown; sporodochium sessile erumpent from the subicle, dark brown to black, pyriform, with long, dark sheathed spines arising from the base and up the sides, 75-150 $\mu$  x 60-100 $\mu$  in size; spines up to 175 or 200 $\mu$  long; conidia brown, elliptical, smooth 9.5-11.5 $\mu$  x 5.5-7 $\mu$ .

From soil: United States: Louisiana

60. *Fusarium* Link 1809 (18)

Conidial layer cushion shaped or somewhat extended without a definite limit. Conidiophores branched. Conidia terminal, single, spindle or sickle shaped, many celled with indistinct cross-walls.

In the arrangement of the species of the genus *Fusarium* the sections as defined by Wollenweber, Sherbakoff, Reinking, Johann and Bailey (62) were used. The key is a modification of that of Sherbakoff (42) changed to fit the forms reported from the soil. The synonymy has been brought up to date following the usage of Wollenweber (61). The descriptions have been taken chiefly from Sherbakoff (42) since these were found most useful in the identification of the species found in the soils examined by the authors.

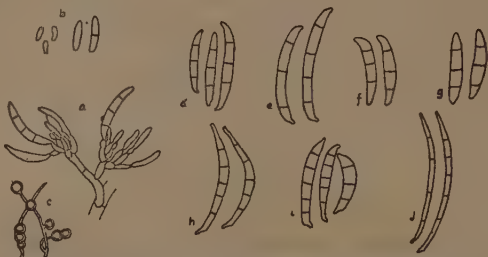


Fig. 81. *Fusarium*. a-conidiophore; b-microconidia; c-chlamydo-sporophores; d-j, macroconidia typical of the sections; d-Elegans; e-Martiella, f-Eupionnotes, g-Ventricosum, h-Gibbosum; i-Discolor; j-Roseum.

KEY TO THE SPECIES OF THE GENUS *FUSARIUM*

- a. Microconidia on aerial mycelium usually present and dominately O-septate, ovoid, fusoid, reniform or pear-shaped.
- b. Conidial walls thin. Macroconidia attenuate at the tip ends, pedicellate; terminal and intercalary chlamydo-sporophores present, color of conidia brownish to salmon; no blue or green color in conidia even as a diffusion from stroma; stroma on artificial media principally vinaceous to lilac.

Section *Elegans*.

- c. Conidiophores simple or only slightly branched.

d. Average macroconidia 36 $\mu$  long.

1. *F. orthoceras*.

dd. Average macroconidia 45.6 $\mu$  long.

2. *F. angustum*.

- cc. Conidiophores typically much branched.
  - d. In plate cultures on neutral potato agar producing exposed and distinct pseudopionnotes.
  - e. Average 3-septate macroconidia 29 $\mu$ . 3. *F. elegantum*.
  - ee. Average 3-septate macroconidia 25 $\mu$ . 4. *F. idahoanum*.
  - dd. No exposed or distinct pseudopionnotes in potato agar plate cultures.
    - e. Macroconidia typically somewhat broader toward apex. 5. *F. blasticola*.
    - ee. Macroconidia typically not broader toward apex.
    - f. Bluish-black sclerotia on potato plugs.
      - g. 3-septate macroconidia up to 45 $\mu$  long. 6. *F. oxysporum*.
      - gg. 3-septate macroconidia up to 55 $\mu$  long. 7. *F. niveum*.
    - ff. Sclerotia not present. 8. *F. lini*
- bb. Conidial walls relatively thick. Macroconidia somewhat truncate or rounded at the tip end, or at least not distinctly attenuate, sometimes slightly constricted at the tip ends, terminal and intercalary chlamydospores present; color of conidia brown-white to golden brown with occurrence of green to green-blue as diffusion from stroma. Section *Martiella*.
  - c. Macroconidia comparatively long and narrow (from 7.4 to 9.3 times longer than broad. 9. *F. radiculicola*.
  - cc. Macroconidia comparatively short and broad (only about 5.5 times longer than broad. 10. *F. solani*.
- aa. Macroconidia on aerial mycelium usually absent or 0-3 or more septate, uniform, comma, spindle to sickle shaped.
  - b. Macroconidia apedicellate. Color type, orange to light salmon.
  - c. Typical pionnotes always present; comparatively slow-growing fungi. Section *Eupionnotes*.
  - d. Conidia typically one septate, three or more septa never present. 11. *F. dimerum*.
  - dd. Conidia typically 3-septate. 12. *F. udum*.
- bb. Macroconidia subpedicellate to pedicellate.
  - c. Chlamydospores present.
    - d. Terminal chlamydospores present; intercalary chlamydospores absent; no true sporodochia; macroconidia wedge-shaped to slightly sickle-shaped, not constricted at the top. Section *Ventricosum*.



- e. Dorsiventrality slight.
  - 13. *F. argillaceum*.
- dd. Intercalary chlamydospores present.
- e. Macroconidia with tip ends much attenuated; stroma typically brown, sometimes carmine.
  - Section *Gibbosum*.
  - f. Substratum, buff to cinnamon and sepia; mycelium from hyaline to brown.
  - g. Conidia typically 5-7 septate.
    - 14. *F. caudatum*.
  - gg. Conidia typically 3-5 septate.
    - 15. *F. lanceolatum*.
  - ff. Substratum white to red.
    - g. Macroconidia strongly arcuate and not prominently broader at the middle.
      - 16. *F. sanguineum*.
    - gg. Macroconidia not much curved and prominently broader at the middle.
      - 17. *F. bullatum*.
- ee. Macroconidia with tip ends somewhat truncate; conidia ochreous to salmon.
  - Section *Discolor*.
  - f. Conidia non- to three-septate, rounded at both ends, of common type, numerous (those of discolor type usually only few).
    - 18. *F. tricothecioides*.
  - ff. Discolor type of conidia common, and nearly the only type present.
    - g. Conidial masses pale cream to pale pink in color, aerial mycelium well developed and nearly white.
      - 19. *F. subpallidum*.
    - gg. Conidial masses of pale orange to dark chocolate-red in color, mycelium from nearly slightly pinkish to dense carmine red.
      - h. Macroconidia mostly 3 septate.
        - i. Average length of 3 septate macroconidia 24 $\mu$ .
        - j. Mycelium and substratum red.
          - 20. *F. sambucinum*.
        - jj. Mycelium and substratum not red.
          - 21. *F. sulphureum*.
      - ii. Average length of 3 septate macroconidia 27 $\mu$ .
        - 22. *F. nigrum*.
      - hh. Macroconidia mostly 5-septate, average length 38 $\mu$ .
        - 23. *F. culmorum*.
- cc. Chlamydospores absent.
  - d. Tip ends of macroconidia gradually attenuate; when free-borne on aerial mycelium, sickle-shaped or none.
    - Section *Roseum*.

- e. Blue sclerotia present.
  - f. Macroconidia typically 5 septate. 24. *F. acuminatum*.
  - ff. Macroconidia typically 3 septate. 25. *F. graminum*.
- ee. Blue sclerotia absent. 26. *F. avenaceum*.
- dd. Tip ends of macroconidia somewhat constricted, conidial walls thick. Section *Saubinetii*.
- e. Macroconidia typically 3 septate. 27. *F. aridum*.

### Section *Elegans*

#### 1. *Fusarium orthoceras* Appel and Wollenweber (42)

Microconidia always greatly in excess, macroconidia ranging from rare to several percent of the total number of conidia, mostly nearly straight, sometimes slightly curved, typically 3-septate,  $36 \times 3.85$  ( $25-40 \times 3.2-4$ ) $\mu$ ; aerial mycelium usually well developed, from white to a tint of olive-buff; substratum, on potato agar rich in glucose, colorless at first, then from russet vinaceous to deep brownish vinaceous; no sporodochia; no pseudopionnotes; no sclerotia.

From soil: Europe (59)

United States: Iowa, Louisiana, New Jersey (52) (53)

#### \*2. *Fusarium angustum* Sherbakoff (42)

Conidia gradually pointed toward apex, from slightly curved to nearly straight or anguiform, usually distinctly pedicellate, mostly 3-septate,  $45.64 \times 3.52$  ( $42-49 \times 3.3-3.6$ ) $\mu$ , often 0- to 5-septate, sometimes 6- to 8-septate; on various agars usually producing thin pseudopionnotes, otherwise very similar to *F. oxysporum*.

From soil: United States: New Jersey (52) (53)

#### \*3. *Fusarium elegantum* Pratt (36)

Microconidia usually present in aerial mycelium, elliptical to oval or slightly curved, typically 0-septate, often 1-septate, averaging  $7.5$  by  $2.8$  ( $4.5-11 \times 2.4-5$ ) $\mu$ , the 1-septate averaging  $12 \times 3.2$  ( $10-17 \times 2.8-4.5$ ) $\mu$ ; macroconidia in aerial mycelium, pseudopionnotes, and sporodochia, slightly curved, typically broader at the middle and in the upper half of their length, somewhat abruptly constricted toward the apex, slightly pedicellate, typically 3- and 4-septate, the 3-septate averaging  $29 \times 4.2$  ( $19-41 \times 3.5-5.5$ ) $\mu$ , the 4-septate averaging  $33 \times 4.6$  ( $26-46 \times 3.6-5.7$ ) $\mu$ , sometimes 2-septate; 1-septate and 5-septate rare; aerial mycelium typically well developed, white; sporodochia and pseudopionnotes, light salmon-orange to salmon-orange on most media, salmon-orange to old rose on Irish potato agar with 10 per cent of glucose; substratum but slightly discolored or not at all, slight-yellow modification on steamed rice; flesh colored to pinkish wart-

like plectenchymic bodies often abundant, especially on steamed melilotus stems; sclerotia often present, dark blue (on steamed potato media); chlamydospores usually present in old cultures, but never abundant, intercalary in the mycelium.

From soil: United States: Idaho (36)

\*4. *Fusarium idahoanum* Pratt (36)

Microconidia always present in aerial mycelium and often in sporodochia and pseudopionnotes, elliptical to oval, sometimes slightly curved, 0-septate, average  $7 \times 2.4$  ( $4.12 \times 1.5-3.5$ )  $\mu$ ; macroconidia slightly curved, typically gradually attenuated toward the apex, slightly pedicellate typically 3-septate, averaging  $25 \times 4.1$  ( $18-40 \times 3-5$ )  $\mu$ , 1-, 2-, and 4-septate common, 5-septate rare; aerial mycelium typically well developed, white at first becoming pink to mallow-purple, often orange-pink when well filled with conidia, frequently developing shades of yellow on Irish potato agar with 10 percent glucose; sporodochia and pseudopionnotes, light orange to orange, substratum (steamed rice) yellow to shades of brown, sometimes, in places, shades of pink to vinaceous; on Irish potato agar with 10 percent of glucose, a rich amber brown.

From soil: United States: Idaho (36)

5. *Fusarium blasticola* Rostrup (42)

Syn. *Fusarium sclerotioides* var. *brevius* Sherbakoff

Macroconidia gradually attenuate toward and more or less pointed at the apex, pedicellate, generally somewhat more distinctly curved near the apex, and broader in the middle or in the upper third of their length, typically 3-septate,  $28.3$  by  $4.3\mu$  ( $19$  to  $39$  by  $3.5$  to  $4.8\mu$ ), rarely 4-septate; chlamydospores observed only in mycelium (intercalary and terminal), and not very common, usually unicellular. Aerial mycelium on hard agars well developed, of medium height (from  $2$  to  $4$  mm.) and density very frequently forming macroscopically observable knots at the hyphal tips, resulting in the production of numerous small sporodochia. Sclerotia not formed. Color of conidial mass somewhat variable, but usually of a tint of pinkish buff; color of substratum varying from nearly colorless when young to cinnamon red, deep vinaceous and dark vinaceous purple. The vinaceous colors were lacking in the culture isolated from the soil.

From soil: United States: Louisiana

\*6. *Fusarium oxysporum* Schlechtendahl (42)

Macroconidia gradually pointed toward apex, nearly cylindrical in middle half of their length, typically not broader toward apex, usually somewhat distinctly pedicellate, 3-septate dominant,  $30.4 \times 4.2$  ( $27.5-34 \times 4.4$ )  $\mu$ , in sporodochia and pseudopionnotes, 4-septate macroconidia frequently, and 5-septate ones rarely present; in mass usually of pinkish buff color; aerial mycelium typically well developed of medium height (from  $3-5$  mm.) and density from white to (in spots on boiled rice) congo pink; substratum, on potato agar rich in glucose, vinaceous lilac, varying from colorless and orange vinaceous to pomegranate purple and vinaceous purple;

plectenchymic sporodochia common on most of the media; bluish-black sclerotia (up to 3 mm. in diameter) constantly present on potato tuber plug and sometimes on different agars.

From soil: United States: California (53), Colorado (53), New Jersey (52) (53), North Dakota (53), Texas (56)

7. *Fusarium niveum* E. F. Smith (46)

Sporodochia numerous, with reduced pionnotes, conidia in mass salmon-colored, macroconidia 3-septate,  $25-55 \times 3.5\mu$ , up to 60 percent, 4- and 5-septate,  $45-60 \times 3.5-5.2\mu$ , up to 15 percent of the conidia present. Sclerotia present, large, blue to green in color. Very like *F. oxysporum* but with larger conidia and larger sclerotia. Wollenweber (58) reports no odor from cultures of *F. niveum* but a slight lilac odor on steamed rice, milk, etc. for *F. oxysporum*. Taubenhaus (46) reports a ripe banana odor with *F. niveum*.

From soil: United States: Texas (46)

8. *Fusarium lini* Bolley (10)

Vegetative hyphae, light colored,  $0.7-3\mu$  in diameter, septate, branching irregularly, ramifying the tissue of the stems and roots of the host. Sporodochia erumpent, compact, slightly raised, distinct but closely grouped upon the stems, pale cream to flesh colored. Sporophores rather short and closely branched, or conidia sometimes arising from wart-like or nearly sessile prominences upon a compact stromatic base. Conidia normally 3-septate, fusiform, slightly curved or flaccate, copiously produced in a bud-like manner from the stroma and from short branches of the sporophores,  $27-38 \times 3-3.5\mu$ . Mycelium on artificial media white, on acid peptone agar a beautiful wine color. Chlamydospores terminal and intercalary.

From soil: United States: Louisiana (53), New Jersey (53), North Dakota (53), Porto Rico (53)

Section *Martiella*

9. *Fusarium radiculicola* Wollenweber (42)

Macroconidia nearly straight near base, slightly curved in upper third of their length, with from somewhat rounded to distinctly constricted apex, slightly pedicellate, mostly 3-septate,  $35.2 \times 4.7$  ( $31-40 \times 4.6-5$ ) $\mu$ ; 0- and 1-septate, microconidia very common, 0-septate measuring  $8 \times 3\mu$ ; chlamydospores common, terminal and intercalary, mostly 0- and 1-septate, 0-septate averaging  $9-10 \times 8.7-8.8\mu$ ; pseudopionnotes typically absent, plectenchymic sporodochia often present; aerial mycelium well developed; color of conidia from white to olive, of substratum from pale yellowish to olive (on agar rich in glucose and on potato tuber plug).

From soil: United States: Idaho (36)

\*10. *Fusarium solani* (Martius) Appel and Wollenweber (6)

Microconidia always present, at least on aerial mycelium, same size and shape as those of *F. martii*. Macroconidia typically somewhat broader in the upper half of their length, with from rounded to slightly constricted

apex, not at all or slightly pedicellate, typically 3-septate,  $29.75 \times 5.5$  ( $27-34.7 \times 5.4-5.8$ ) $\mu$ , sometimes 4-septate, rarely 5-septate; aerial mycelium from poorly to well developed, from white to olive-buff; substratum, on potato agar rich in glucose, olive-buff with a green-blue tinge.

From soil: England (15)

United States: California (53), Colorado (53), Hawaii (53), Louisiana (53), Maine (53), New Jersey (52) (53), Texas (53)

#### Section *Eupionnotes*

##### \*11. *Fusarium dimerum* Penzig (42)

Conidia lunar, somewhat pedicellate, typically 1-septate,  $13 \times 3.3$  ( $12.5-13.5 \times 3.3-3.4$ ) $\mu$ , often also 0-septate, rarely 2- or 3-septate, borne singly on the mycelium or forming a more or less continuous slimy layer, from hyaline to cinnamon-buff on glucose agar; mycelium from hyaline to about the color of the conidial masses; chlamydospores intercalary, in mycelium.

From soil: United States: Idaho (36)

##### 12. *Fusarium udum* (Berkeley) Wollenweber (42)

Conidia dorsiventral, usually somewhat broader toward the slightly rounded apex, apedicellate, typically 3-septate,  $33-45 \times 3.5-4\mu$ , non- to two-septate, very rare when mature, 4- and 5-septate, of from light vinaceous cinnamon to orange-cinnamon hues on agars rich in glucose; chlamydospores usually found only in old cultures, terminal and intercalary, in conidia, in the tips of sterigmata, and in mycelium, often of dense orange color, 0-septate,  $6 \times 5.5\mu$ ; aerial mycelium present only near margin of colony growth, very loose, short, hyaline, substratum colorless or approaching the color of the conidia.

From soil: Europe (59)

United States: Louisiana

#### Section *Ventricosum*

##### 13. *Fusarium argillaceum* (Fries) Saccardo (61)

Syn. *Fusarium ventricosum* Appel and Wollenweber (42)

*Fusarium cuneiforme* Sherbakoff (42)

Conidia only slightly dorsiventral, more or less wedge-shaped, broader toward the base, with apex somewhat rounded, apedicellate, typically 3-septate,  $34.7 \times 5.6$  ( $30-41 \times 5.6-6$ ) $\mu$ , often 0- to 2-septate, in false balls, from hyaline to cream colored; no sporodochia; aerial mycelium hyaline, in a high tuft in center, and short, distinctly zonate, outside; chlamydospores from smooth to very distinctly warted sometimes surrounded with a gelatinous capsule, terminal only, typically unicellular  $8.2 \times 7.6$  ( $7.6-8.5 \times 7.3-8.1$ ) $\mu$ .

From soil: United States: Louisiana

#### Section *Gibbosum*

##### 14. *Fusarium caudatum* Wollenweber (42)

Conidia with from parabolic to ellipsoid dorsal curve, conspicuously broader at the middle, with very long, narrow, whip-like apex, prominently



pedicellate, typically 5- to 7-septate; measuring on the average, 5-septate  $48 \times 3.4.5$  ( $40-80 \times 3.0-4.5$ ) $\mu$ ; 7-septate,  $64.7 \times 4.6$  ( $57-69 \times 4.4-4.8$ ) $\mu$ ; rarely in pseudopionnotes; typically in small aplectenchymic sporodochia, tinted from cream-buff to cinnamon, clay and Saccardo's amber in a plate culture on a potato hard agar, rich in glucose; chlamydospores brown, intercalary, always present in greater or less abundance; aerial mycelium well developed, high, uniform, medium dense, from hyaline when young to sepia in old cultures, mostly from brownish to dresden brown; substratum, on potato agar rich in glucose, from pinkish buff, when young to ochraceous tawny and snuff brown shaded to sepia in very old cultures.

From soil: Canada (53)

United States: California (53), Colorado (52) (53), Iowa (53), New Jersey (52) (53), Porto Rico (53), Texas (52) (53)

\*15. *Fusarium lanceolatum* Pratt (36)

Conidia typically in pseudopionnotes, but also in aerial mycelium and sporodochia from nearly straight to strongly curved, usually distinctly pedicellate, typically 3-, 4-, and 5-septate, 6- and 7-septate common, higher septations rare, the 3-septate averaging  $34 \times 3.5$  ( $22$  to  $52 \times 2.5-5$ ) $\mu$ , the 4-septate,  $40 \times 3.8$  ( $22-60 \times 2.8-5$ ) $\mu$ , and the 5-septate  $48 \times 4.1$  ( $36-70 \times 2.8-5.7$ ) $\mu$ ; aerial mycelium scantily developed, white when present, to dark maroon when well filled with conidia; sporodochia and pseudopionnotes ochraceous-orange at first, becoming dark maroon, often brighter shades of red to Brazil red; substratum on steamed potato often a bright orange, yellow modification on rice becoming brown with age; chlamydospores singly and in chains.

From soil: United States: Idaho (36)

Sub-section *Ferruginosum*

\*16. *Fusarium sanguineum* Sherbakoff (42)

Conidia typically strongly arcuate, gradually pointed toward apex, distinctly pedicellate, 3- to 5-septate, 3-septate conidia averaging  $33.5 \times 3.6$  ( $24-37 \times 3.4-3.8$ ) $\mu$ , and 5-septate averaging  $45.2 \times 4.1$  ( $40-47 \times 3.9-4.2$ ) $\mu$  single, in from small to medium-sized (up to 2 mm. in diam.) sporodochia and in pseudopionnotes, the latter form of fructification dominant on most media, especially on agars; chlamydospores almost always present, intercalary in conidia and in mycelial threads, borne singly, in chains and in clusters; aerial mycelium seldom well developed and then from white to different shades of pink, on various agars mostly absent, leaving exposed pseudopionnotes of ox-blood red color.

From soil: United States: Idaho (36)

17. *Fusarium bullatum* Sherbakoff (42)

Conidia typically somewhat less arcuate than the other species of the same section, less pointed toward the apex, and broader, usually distinctly pedicellate, mostly 5-septate,  $42 \times 4.3$  ( $31-47 \times 4.1-4.9$ ) $\mu$ , from pale cream to salmon in color; chlamydospores intercalary in mycelium mostly in chains and from small to large clusters; aerial mycelium nearly always well

developed, of uniform medium height and density, nearly pure white in color, substratum on various agars from colorless to a tint of light buff.

From soil: United States: Alaska (53), Louisiana (53), Maine (53), New Jersey (52)

\*18. *Fusarium trichothecioides* Wollenweber (25)

Conidia of common and discolor types, the former predominating and under ordinary cultural conditions occurring almost exclusively, mostly 1-septate,  $16 \times 4.6$  ( $14-17 \times 4.2-5.4$ ) $\mu$ , often 0- to 3-septate, seldom 4- or 5-septate, 6-septate rare; sporodochial conidia sickle-shaped, 3- to 5-septate,  $24-42 \times 4.5-5.5\mu$ . *F. trichothecioides* can be recognized at once by color and appearance of its powdery masses of spores produced on aerial mycelium. Chlamydospores few and not prominent.

From soil: United States: Idaho (36)

\*19. *Fusarium subpallidum* Sherbakoff (42)

Conidia sickle-shaped, typically abruptly constricted at apex, slightly pedicellate to papillate, somewhat broader in the middle, mostly 3-septate,  $29.1 \times 5.53$  ( $28-32.5 \times 5.4-5.8$ ) $\mu$ , 3- and 4-septate common, 6- and 7-septate very rare; chlamydospores common, mostly in long chains; aerial mycelium well developed; plectenchymic sporodochia (up to 3 mm. in diam.) common; color of aerial mycelium from white to sea foam yellow and honey yellow; color of substratum, on agars rich in glucose, mostly from chamois to raw sienna and antique brown in some old cultures; color of conidia, in mass, commonly from pinkish-buff to pale orange, sometimes from green to blue.

From soil: United States: Idaho (36)

\*20. *Fusarium sambucinum* Fuckel (42) (61)

Syn. *Fusarium discolor* Appel and Wollenweber (42)

Conidia for greater part of their length of nearly even diameter, sickle-shaped, gradually attenuated, often somewhat suddenly constricted at the apex, pedicellate, mostly 3-septate, measuring  $24.2 \times 4.7$  ( $22-26 \times 4.5-4.9$ ) $\mu$  on aerial mycelium, in pseudopionnotes, and in plectenchymatic sporodochia; by the presence of very large (up to 1.2 centimeters in diam.), warty, plectenchymic bodies (producing conidia or remaining sterile) of a pale pinkish buff with spots of darker color; chlamydospores scant, not in long chains; aerial mycelium from poorly to well developed, from pale pink-buff to ochraceous orange and Eugenia red; color of substratum, on agars rich in glucose, from pale salmon at an early stage and warm sepia in old cultures to tyrian and ox blood; color of conidia mostly from light ochraceous salmon to ochraceous buff.

From soil: United States: Idaho (36)

\*21. *Fusarium sulphureum* (Schlechtendahl) Wollenweber (42) (61)

Syn. *F. discolor* var. *sulphureum* (Schlecht) App. & Wr.

Differs from *F. sambucinum* by the absence of red color in mycelium and substratum and by entirely exposed pseudopionnotes on various agars.

From soil: Europe (61)

\*22. *Fusarium nigrum* Pratt (36)

Conidia in aerial mycelium, pseudopionnotes, and sporodochia, slightly curved, somewhat abruptly constricted toward the apex, typically broader at the middle and in the upper half of their length, typically 3- and 4-septate, the 3-septate averaging  $27.5 \times 4.7$  ( $18-38 \times 3.6-5.9$ ) $\mu$ , the 4-septate averaging  $31 \times 5$  ( $21-43 \times 3.6-6$ ) $\mu$ ; aerial mycelium typically well developed, from white to reddish brown, often nearly ox-blood red, the appearance of shades of red and brown signaling the development of chlamydo-spores; on Irish potato agar with 10 percent of glucose, various shades of red and brown, discoloring the media from amber-brown to nearly black; sclerotia-like bodies, consisting of masses of mycelium; conidia and chlamydo-spores, typically present on starchy media and steamed melilotus stems, from ox-blood red to sepia brown and black; sporodochia salmon-orange to ochraceous-orange and buckthorn-brown (on string bean agar). Chlamydo-spores terminal and intercalary, singly and in chains and groups.

From soil: United States: Idaho (36)

23. *Fusarium culmorum* (W. G. Smith) Saccardo (42)

Syn. *Fusarium rubiginosum* Appel and Wollenweber (6)

Conidia for a greater part of their length of an even diameter mostly 5-septate,  $38.5 \times 5.85$  ( $37-40 \times 5.3-6.2$ ) $\mu$ , somewhat suddenly constricted at apex; pedicellate, of distinctly ochraceous orange color under microscope; chlamydo-spores of more or less common occurrence on mycelium and in conidia, not in long chains; aerial mycelium well developed, high (up to 1 cm. and more), very loose, at first from white to pinkish cinnamon, and then to jasper and Eugenia red; substratum, on potato agar rich in glucose, of from spectrum red to carmine pomegranate purple, with more or less brick red color; color of conidia in mass from cinnamon and light ochraceous to mikado brown and warm sepia; sporodochia minute, separate or converging into pseudopionnotes.

From soil: United States: Idaho (36), Iowa, Louisiana, Utah

Section *Roseum*

\*24. *Fusarium acuminatum* (Ellis and Everhart) Wollenweber (42)

Conidia scattered, in sporodochia or in pionnotes, orange in mass. Conidia average as follows: 5-septate,  $40-70 \times 3-4.5\mu$ ; 4-septate (less common)  $30-60 \times 3-4.5\mu$ ; 3-septate,  $20-45 \times 2.75-4.25\mu$ . Conidia of 0-, 1-, 2-, 6-, and 7-septations are occasionally found. Sub-normal small conidia may be mistaken for conidia of the section *Discolor* but normal sporodochia develop on repeatedly whorl-like branched conidiophores, giving the characteristic conidia of the section *Roseum*. The conidia show in side view hyperbolic or parabolic curves, in contrast to *F. metacroum* App. and Wr., the conidia of which are as a rule more nearly straight. Blue globose sclerotia, 50-70 $\mu$  thick, occur and form striking contrast to the carmine plectenchymatic thallus on starchy media, such as steamed potato tubers. Both blue and carmine are basic modification of the fungus while yellow (on rice) is the acid one, turning blue to purple violet with the addition of alkali.

From soil: United States: Idaho (36)

\*25. *Fusarium gramineum* Corda (37)

Sporodochia erumpent, diffuent, golden yellow, interior white. Conidiophores branched, thin, conidia cemented, thin, spindle-shaped, straight, then curved, very pointed and pale colored, with 3-septa,  $30-40 \times 3.5\mu$ .

From soil: Europe (61)

\*26. *Fusarium avenaceum* (Fries) Saccardo (42)

Syn. *Fusarium subulatum* Appel and Wollenweber

Conidia slightly elliptically curved, typically of nearly even diameter for the greater part of their length, very gradually attenuate toward both ends, slightly pedicellate, typically 5-septate,  $58 \times 3.64$  ( $48-65 \times 3.4-3.85$ ) $\mu$ , usually numerous, sometimes converging sporodochia ( $\frac{1}{4}$  to 1 mm. in diam.), produced near substratum and lower aerial mycelium, from pink-flesh to apricot-buff and from coral red to brick red in color, with dark shades in old moist cultures; chlamydospores absent; aerial mycelium typically present, at first hyaline, then testaceous color and other hues of red, on various agars from vinaceous tawny and madder brown to pomegranate purple and Eugenia red.

From soil: Cosmopolitan (42)

Section *Saubinetii*\*27. *Fusarium aridum* Pratt (36)

Conidia in aerial mycelium, pseudopionnotes, and sporodochia, slightly curved, typically broader in the upper half of their length, usually suddenly constricted at the apex, slightly pedicellate, typically 3-septate, averaging  $27 \times 4.2$  ( $18-36 \times 3-5$ ) $\mu$ ; 1-, 2- and 4-septate usually present, the 4-septate rare; aerial mycelium typically well developed, white at first, becoming pink to vinaceous; substratum on steamed potato plug, often vinaceous to Vandyke red; on Irish potato agar with 10 percent of glucose vinaceous-purple to carmine; steamed rice shades of yellow and brown; sporodochia and pseudopionnotes, salmon-orange to light-orange. Chlamydospores not observed.

From soil: United States: Idaho (36)

61. *Myrothecium* Tode 1790 (37)

Conidial layer, shield or cushion shaped, black, surrounded at the edge by fine hyaline cilia. Conidiophores short rod-shaped. Conidia very small, ovoid or cylindric.

\*1. *Myrothecium roridum* Tode (37)

Sporodochium shield-shaped, then confluent and sessile, black, with a white rim, 2-6 mm. in diameter. Conidiophores unbranched or forked, bush-like,  $30-40\mu$  long,  $2\mu$  wide. Conidia cylindric truncated at both ends, with 2-oil drops, smoky olive-green, 8-10 seldom  $14\mu$  long, by  $2\mu$  thick.

From soil: United States: North Dakota (53)



Fig. 82. *Myrothecium*. (After Lindau).



## VII. MYCELIA STERILIA

62. *Rhizoctonia* De Candolle 1815 (18)

Sclerotia without definite form, often grown together, horny-fleshy, with thinner undifferentiated edges, frequently imbedded in the mycelium and bound together by mycelial strands. Fructification unknown.

\*1. *Rhizoctonia solani* Kuhn, *Corticium vagum* Berkeley & Curtis (11)

Vegetative mycelium saprophytic in the soil and in wood in contact with the ground, and parasitic as in the *Rhizoctonia solani* stage in underground portions of various plants and forming at their surface underground minute sclerotia; fructification a thin arachnoid, perforate membrane more or less separable, pale olive-buff to cream color; in structure 60-100 $\mu$  thick, composed of a few loosely interwoven hyphae running along the substratum and sending out short branches which bear basidia; hyphae in contact with the substratum may be slightly brownish, hyaline elsewhere, not incrustated, not nodose-septate, up to 6-10 $\mu$  in diameter with branches smaller; basidia not forming a compact hymenium, 10-12 x 7.5-11 $\mu$ , with 4-6 sterigmata 6-10 $\mu$ , long and more or less swollen towards the basidium; spores hyaline, even, flattened on one side 8-14 x 4-6 $\mu$ .

From soil: United States: Idaho (36)



Fig. 83. *Rhizoctonia*. (After Burt).

## EXCLUDED SPECIES

The following species which have been reported from the soil were not included in the key either because of insufficient knowledge concerning them or because it was felt they did not come within the scope of the paper.

1. *Aphanomyces laevis* de Bary. A soil saprophyte (26)
2. *Sphaeronema fagi* Oudemans. Isolated from decaying leaves (26)
3. *Coccospora agricola* Goddard (20)
4. *Sachsia albicans* Bay (26)
5. *Myceliophthora sulphurea* Goddard (20)
6. *Geotrichum candidum* Link. On barren soil (26)
7. *Papulaspora pannosa* (?) (53)
8. *Corethrospis paradoxa* Corda. On soil (26)
9. *Dematium pullulans* de Bary (53)
10. *Bispora pusilla* Saccardo. From decaying wood in soil (26)
11. *Torula lucifuga* Oudemans (26)
12. *Willia anomala* Hansen (26)
13. *Willia saturnus* Klocker (26)
14. *Saccharomycopsis capsularis* Schonning (26)
15. *Hansenia apiculata* Lendner (26)
16. *Saccharomyces glutinus* Fresenius (5)
17. *Allescheriella nigra* (?) (45)



18. *Trichoderma flavus* Abbott (2) *Trichoderma glaucum* Abbott was listed in Louisiana Bulletin 194 under this name by mistake.
19. *Scopulariopsis roseum* (16)

#### ADDITIONAL GENERA

A number of fungi have been reported from the soil by genus name only. A list of these for which no species are listed elsewhere in this paper follows:

1. *Hypoderma* (*Hyphoderma*?) Hawaii (53)
2. *Sordaria* Texas (53)
3. *Rhinotrichum* New Jersey (53), Oregon (53)
4. *Zygodesmus* Porto Rico (53), Oregon (52)
5. *Melanconium* New Jersey (53)
6. *Hyalopus* Louisiana (2). Later identified as a species of *Gliocladium*, and reported in this paper as *G. atrum*, n. sp.
7. *Oedocephalum* Louisiana (2). Later identified and reported in this paper as *Cunninghamella verticillata* Paine.
8. *Thielavia* Louisiana (2)
9. *Marasmius* Louisiana (2)
10. *Monotospora* Louisiana (2). Later identified as a species of *Acremoniella*, and reported in this paper as *A. brevia*.
11. *Epicoccum* Louisiana (2)
12. *Pestalozzia* Louisiana (2)
13. *Brachysporium* Louisiana (2). Later identified and reported in this paper as *Spondylocladium xylogenum* Smith.
14. *Periola* England (16)
15. *Monascus* Idaho (36)
16. *Rhapalomyces* North Dakota (8)
17. *Colletotrichum* North Dakota (8)
18. *Ozonium-Sepedonium*. See Dale (16)
19. *Isaria* Iowa (3)
20. *Sclerotium* (52)

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# THE PROTOZOAN FAUNA OF THE RUMEN AND RETICULUM OF AMERICAN CATTLE

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This paper is chiefly a report of a survey of the protozoan fauna of the rumen and reticulum contents of twenty-six cattle slaughtered in the abattoir at Iowa State College. The writers are very much indebted to Professor M. D. Helser and other members of the Animal Husbandry Department of this institution for their kindly interest in the subject, shown by their co-operation in assisting and permitting us to secure the material for the examinations. The survey was made as an essential preliminary to an investigation of the physiological rôle of these protozoa in their hosts.

The existence of a wealth of protozoan life in the rumen and reticulum of cattle was first noted by Grube and Delafond (1843). They likewise noted an almost equally remarkable protozoan fauna in the coecum of the horse, and found protozoa in the intestine of the dog and pig. Their descriptions of four ciliates from the cow were sufficiently clear for Eberlein (1895, p. 234) and Sharp (1914, p. 45) to recognize in them the genera *Ophryoscolex*, *Diplodinium*, *Entodinium*, and *Isotricha*.

Since Grube and Delafond, many papers have appeared which deal wholly or in part with various protozoa from ruminants. For a complete review of the subject up to 1914, the reader is referred to Sharp (1914). The more outstanding papers during this interval were those by Stein (1858, 1859, 1867), Schuberg (1888, 1891), Fiorentini (1890), Eberlein (1895), and Braune (1913). Sharp (1914) published on the morphology of *Diplodinium ecaudatum* and its varieties. He gave for the first time an accurate detailed description of the morphological organization of this ciliate, which is considered by many to be one of the most complicated cells of which we have any knowledge. His most noteworthy contribution, however, was the discovery of a neuro-motor apparatus, which suggested a coordinating system similar in function to the nervous system of metazoan animals. Awerinzew and Metafowa (1914) gave an account of five supposedly new species from oxen slaughtered in St. Petersburg. As we shall see later, four were synonyms of already described species, and the fifth may possibly be new, although the creation of a new genus for it was not justifiable. Cunha (1914) published on the ciliates found in the stomachs of Brazilian cattle. There is nowhere in the literature, however, a complete compilation of all the protozoa described from the rumen and reticulum of the cow, although Buisson (1923) mentions and figures most of the ciliates.

The protozoan fauna of the first two divisions of the stomachs of cattle belong to three classes (or sub-phyla), the Sarcodina, Mastigophora, and Infusoria, and embrace thirteen valid genera and thirty-nine species and varieties. In this paper, three hitherto undescribed species of ciliates and one previously known species of amoeba not hitherto reported from the cow are presented.

## CLASS SARCODINA

Liebetanz (1910) and Braune (1913) established the presence of one member of this class, *Endamoeba bovis*, in cattle. We have found one other which is described below.

Family AMOEBIDAE Bronn 1859

Genus *VAHLKAMPFIA* Craig 1913

This genus was established for an amoeba cultivated by Musgrave from the faeces of a patient suffering from amoebic dysentery. Its shape is oval or spherical when not in motion, and very irregular when the amoeba is moving. There is little distinction between ectoplasm and endoplasm in small amoebae, but in larger amoebae the ectoplasm is more refractile. The nucleus is well defined in living organisms, appearing as a circular, refractile body surrounded by a narrow hyaline halo; the nuclear membrane is indistinct. Stained nuclei show a large central karyosome filling at least two-thirds of the nucleus, and an indistinct nuclear membrane with a zone of fine chromatin granules. The amoeba has one contractile vacuole. It has no flagellated stage in its life history.

1. *Vahlkampfia lobospinosa* (Craig 1912) Craig 1913

This is normally a free-living amoeba, but Craig (1925) believes that it may exist in the human intestine and live there for a limited period, as he has found what he believes to be the vegetative form of this amoeba in uncontaminated human faeces. The writers found it present in considerable numbers in the rumen of one cow. Whether they were ingested with the drinking water, or had succeeded in establishing themselves there as accidental parasites is not known.

Specimens studied from cultures showed nuclei with karyosomes somewhat smaller than those figured by Craig, and the nuclear membrane was more distinct, with very little granulation (Fig. 1). Fine linin threads radiated from karyosome to nuclear membrane. Several large individuals with two, three, and four nuclei were found. No contractile vacuole was present. In locomotion the clavate shape was often temporarily assumed. Size: uninucleate forms,  $18 \times 10\mu$ ; multinucleate forms,  $24 \times 18\mu$ . Cysts with one or two nuclei (Fig. 2). Size of cysts 7 to  $11\mu$ .

Family ENDAMOEBIDAE Calkins 1926

Genus *ENDAMOEBIA* (Leidy 1879) Stiles and Boeck 1923

Type: *Endamoeba blattae* Leidy 1879, an inhabitant of the intestine of the cockroach. The nucleus possesses a small chromatic endosome surrounded by a halo. The nuclear membrane is beaded with chromatin granules. There is a linin reticulum on which chromatin granules may be suspended between the halo and the nuclear membrane. No contractile vacuole. All parasitic.

2. *Endamoeba bovis* (Liebetanz 1910) Braune 1913

We have encountered specimens of this amoeba several times in small numbers, but were unable to affix them to the slide for staining purposes.

The nucleus is similar to the *Endamoeba histolytica* type in unstained specimens. Cysts have not yet been described. Size about 20 $\mu$ . For figure see Braune (1913).

# CLASS MASTIGOPHORA

Liebetanz (1910) deserves credit for the discovery of flagellates in the stomachs of cattle. He describes and figures fourteen species of them. His results, however, are open to very serious criticism, as he used the dry fixation method which is unreliable for careful morphological studies of flagellates. (*Vide* Braune, 1913, p. 119). All of his flagellates are the type with one flagellum, while Braune (1913) found that three out of the five species which he found in the cow had three or more flagella. As an example of one of the errors into which Liebetanz was led, we point out his Fig. 6, which he calls *Sphaeromonas communis*. It was undoubtedly a *Trichomonas* with an undulating membrane which he actually saw. Then there is the further likelihood that many of his species were free-living protozoa which the cattle had swallowed with their drinking water. His Dinoflagellates, *Peridinium tabulatum* and *Amphidinium lacustre* were undoubtedly of this nature because, as he himself states, they possessed neither flagella nor movement.

For the sake of adding completeness to this work we herewith list the species of flagellates described by Liebetanz (1910), recognizing their validity as flagellate parasites of cattle only insofar as they have been restudied and their validity confirmed by Braune (1913).

1. *Sphaeromonas communis*
2. *Sphaeromonas minima*
3. *Sphaeromonas maxima*
4. *Oikomonas communis*
5. *Oikomonas minima*
6. *Cercomonas rhizoidea communis*
7. *Cercomonas rhizoidea minima*
8. *Cercomonas rhizoidea maxima*
9. *Piromonas communis*
10. *Piromonas minima*
11. *Piromonas maxima*
12. *Mastigamoeba bovis*
13. *Peridinium tabulatum*
14. *Amphidinium lacustre*

The work of Braune (1913) bears the mark of thoroughness. We have found three of his five species in the cow, and one in the sheep.

Family MONADIDAE Kent 1880

Genus *MONAS* Mueller 1773

Small, spherical, colorless forms possessing one flagellum, or one long and one extremely short flagellum.

3. *Monas communis* (Liebetanz 1910) Braune 1913

Syn. *Sphaeromonas communis* Liebetanz 1910

Spherical, about four microns in diameter. Nucleus at anterior end. Flagellum arises from basal granule connected with the nucleus by a rhizo-

plast. Flagellum directed backwards during locomotion. It is a weak swimmer. Mouth and contractile vacuole not known to be present. We have found this flagellate in a few cattle.

Genus *PIROMONAS* Liebetanz 1910

Small, pear-shaped, colorless forms. Anterior end in comparison to posterior end is more reduced and rounded. The nucleus lies just in front of the middle of the body, beside a lateral indentation of the body. The flagellum arises from a basal granule near the nucleus and wraps itself about the long axis of the body during locomotion.

4. *Piromonas communis* Liebetanz 1910

Body about  $8\mu$  in length, flagellum  $23\mu$ . Although Braune reports that it is frequently found in European cattle, we have not found it.

Family TETRAMITIDAE Bütschli

Genus *EUTRICHOMASTIX* (Kofoid and Swezy 1914) Stiles and Hassal 1925

(nec *TRICHOMASTIX* Blochmann 1884)

Three anteriorly directed flagella, one larger posteriorly directed flagellum; all four originate in an anterior blepharoplast. Adjacent to the latter are the crescentic cytostome and the nucleus. From the latter the slender axial axostyle passes to the posterior end.

5. *Eutrichomastix ruminantium* Braune 1913

The morphology of this species does not correspond exactly with the generic characters stated above, for the posteriorly directed flagellum and cytostome have not been observed. There is an abundance of dark staining reserve food substance in the cytoplasm, which makes internal structures difficult to observe. We have found this flagellate in sheep, but not in cattle. Body length about  $8\mu$  (Fig. 4)

Genus *TRICHOMONAS* Donné 1837

Three, four, or five anteriorly directed flagella; undulating membrane bounded externally by flagellum running from anterior toward posterior end. Axostyle present.

6. *Trichomonas ruminantium* Braune 1913

This species was observed by us in both sheep and cattle. It sometimes occurs in large numbers. There are three long anterior flagella. Size in stained preparations  $7$  to  $9\mu$  (Fig. 5).

We have succeeded in cultivating this flagellate in the Hogue ovomucoid medium which is so useful for the cultivation of the flagellates of the human intestine (Hegner and Becker 1922). In cultures it increases rapidly. It advances through the mass of bacteria by thrusting its flagella forward into the mass and lashing them backwards. The undulating membrane, with the rotating of the body on its long axis, serves to clear a tunnel along the path of the flagellate, so that it is able to turn about easily and even

reverse its direction. We have never observed any flexion or active movement of the axostyle, as has been described by some writers for other *Trichomonas*. It seems to serve as an organ for giving the body rigidity.

Family POLYMASTIGIDAE

Genus *CALLIMASTIX* Weissenberg

Body elongated, egg-shaped; nucleus anterior to the middle; in anterior region a number of basal granules, from each of which a flagellum united with the others arises and passes posteriorly on one side. The flagella vibrate as a unit.

7. *Callimastix frontalis* Braune 1913

As described by Braune (1913), this species has a clear, granule-free, disk-shaped area (*Stirnfeld*) in the anterior part, on the margin of which lie 12 basal granules, which give rise to 12 flagella (Fig. 3). These flagella are united and function as a unit. Outside of the *Stirnfeld*, the endoplasm is filled with deeply staining granules. The nucleus is small and lies just back of the *Stirnfeld*. Length of body about 12 $\mu$ ; flagella, 30 $\mu$ .

We have found this flagellate in two instances. It is not as lively in its movements as *Eutrichomastix* and *Trichomonas*, and is easily overlooked. It moves about jerkily, and in a circle.

CLASS INFUSORIA

Family ISOTRICHIDAE Bütschli 1888

Genus *ISOTRICHA* Stein 1859

This genus is difficult to define on account of the wide disagreement in the interpretation of its structures. Perhaps it would be best to give in general Schuberg's (1888) definition. Body egg-shaped, dorsoventrally compressed; surface covered with long cilia which run in longitudinal rows, so that the striations meet along a line running from the mouth (at or near the anterior end) to the posterior end (this line is the so-called *Mundnaht*); at the posterior end is a peculiar structure which Stein designated as the *Afterspalte* or anal split; gullet long, at anterior end, with fine spiral striations; several contractile vacuoles in anterior body region; micronucleus close beside macronucleus, both enclosed in a capsule which is joined to the body wall by connecting strands, or *Kernsteile*. In locomotion the posterior end is forward, the mouth lying in the end away from the direction of movement.

The controversial points in *Isotricha* are the *Afterspalte*, the *Mundnaht*, and the *Kernsteile*. The morphology of this ciliate needs a complete re-investigation. We have, however, studied it in sufficient detail to feel sure that the *Afterspalte* is, as Schuberg claimed, merely a thickening of the "dermal" layers. There is, however, as Braune (1913) described, also a weakly bow-shaped series of fibrils in the region of the body lying near the so-called *Afterspalte*. Braune (1913) identified this structure with the *Afterspalte* of Stein and Schuberg. As a matter of fact, both structures are present (see Fig. 6). The so-called *Mundnaht* is short, terminating long before it has reached the mouth. In fact, we feel doubtful if the so-called mouth is a mouth at all. We have never seen any solid food in the endo-



plasm, which is remarkably clear. The existence of a ciliated tube leading from the circular surface opening does not necessarily imply a gullet, for it is plain that it may represent merely an invagination of the surface layers, vital membrane, cuticle, cilia, and all. And this invagination may be in the region of the primitive anus! If the line where the striations meet is comparable to the *Mundnaht* of, for example, *Chilodon*, it should end at the so-called mouth. Since it does not, it seems more likely that the mouth has disappeared as it shifted posteriorly, and that the organism became a saprophyte. This view is certainly a saner one than one which presumes either a physiological reversal of the organism, or the shifting of the mouth clear to the posterior end. For the latter to be true, the anus must have shifted to the front! We fail, however, to recognize in the *After-spalte* other than a thickening of the cuticle and vital membrane—similarly Schuberg. We have seen Braune's so-called *Afterfibrillen* arising from basal granules and can see in them more likelihood that they represent a part of a neuromotor apparatus, than that they have anything to do with an anal structure.

#### 8. *Isotricha prostoma* Stein 1859

So-called mouth at the end which is backwards in locomotion, lying somewhat toward "ventral" side (Fig. 6). Size 80 to 195 $\mu$  x 53 to 85 $\mu$ . We have frequently found this ciliate in large numbers.

#### 9. *Isotricha intestinalis* Stein 1859

So-called mouth lying in a surface indentation somewhat removed from the end which is backwards in locomotion (Fig. 7). Size 97 to 130 $\mu$  x 68 to 88 $\mu$ . We have found this species occasionally.

#### Genus *DASYTRICHA* Schuberg 1888

Body oval, flattened dorsoventrally; body covered with cilia arranged in longitudinal spirals. Nucleus without supporting *kernstiele*. Striations in so-called pharynx are longitudinal, in contrast to those in *Isotricha* which are said to be spiral (we believe them to be longitudinal in pharynx of *Isotricha* also). There is considerable controversy over the structure of this ciliate also; e.g., Braune would unite this genus with *Isotricha*. We believe it legitimate to retain the genus on the spiral striations of the body alone.

#### 10. *Dasytricha ruminantium* Schuberg 1888

Only one species of above described genus (Fig. 8). Length 50 to 75 $\mu$ ; width 30 to 40 $\mu$ . We have found this ciliate frequently and in large numbers.

#### Family BÜTSCHLIIDAE

#### Genus *BÜTSCHLIA* Schuberg 1888

Small holotrichs, more or less ovoid in shape; mouth at anterior end, surrounded by cilia longer than those over the entire remaining body surface; ectoplasm extremely thick at anterior end, but thin over remainder

of body; nucleus more or less spherical, but micronucleus not yet observed. There is always a mass of crystalline refractile concretions, the *Concrementhaufen*.

11. *Bütschlia parva* Schuberg 1888

Anterior end of body sharply truncated, body otherwise rounded; mouth situated in the middle of the anterior end leads into a short conical pharynx; no anus present; cilia over body arranged in longitudinal rows; contractile vacuole absent according to some authors, occasionally present according to others (Fig. 9). Length, 30 to 50 $\mu$ ; width, 20 to 30 $\mu$ .

We have not found this or any other species of *Bütschlia* in our survey. Sharp (1914, p. 51) implies that he observed *B. parva* from cattle slaughtered in California.

12. *Bütschlia neglecta* Schuberg 1888

This species resembles *B. parva*, except that the posterior end is somewhat pointed and provided with four indentations, so that a cross section through the posterior end would present the form of a cross with the inner angles rounded. Length, 40 to 60 $\mu$ ; width, 20 to 30 $\mu$ .

13. *Bütschlia lanceolata* Fiorentini 1890

So far as we can learn, this species has been observed by no one since Fiorentini. The body is lanceolate in form, presenting in the upper fifth of the body a stricture in the form of a collar surmounted by very fine cilia. At the anterior end is an oval aperture followed by a pharynx which extends backwards a third of the length of the body. The nucleus is large and pale. A mass of calcareous concretions is located in the posterior part of the body. Size 48 by 20 $\mu$ .

Family OPHRYOSCOLECIDAE Stein 1858

Genus *ENTODINIUM* Stein 1858

Members of this genus possess no cilia other than an adoral zone of brush-like membranelles. The mouth occupies most of the truncated anterior end; gullet passes posteriorly from the mouth; anus small, at posterior end. There is in all known species one macronucleus, one micronucleus, and one contractile vacuole. The surface is generally finely striated longitudinally, the striations being difficult to observe.

14. *Entodinium bursa* Stein 1858

The body is said to be flattened dorsoventrally; but if by homology we use the terms which Sharp (1914) applied to *Diplodinium ecaudatum*, it would be more correct to say that it is flattened laterally. The anterior end is sharply truncated. The large macronucleus extends at least four-fifths the length of the body. The micronucleus is closely adpressed against the macronucleus. One contractile vacuole is present, usually represented to be on side opposite the nucleus. We found the vacuole more often in the position figured (Fig. 10). Surface of body conspicuously longitudinally striated. According to Schuberg the length is 55 to 114 $\mu$ , width 37 to

78 $\mu$ . We find that the average size is about 80 by 60 $\mu$ . Fig. 10 represents one of the smaller sizes.

We found this species to be present in all but a few of the cattle examined.

15. *Entodinium minimum* Schuberg 1888

Flattened laterally (cf. *E. bursa*); dorsal margin of the body (nearest the nuclei) strongly convex; ventral margin almost straight, or slightly curved; body faintly longitudinally striated (not shown in our figure); average size 40 x 22 $\mu$ . We found this ciliate occurring more frequently than any other in American cattle (Fig. 15).

16. *Entodinium caudatum* Stein 1858

This *Entodinium* distinguishes itself by the presence of three caudal prolongations of the body; one, a long, narrow, dorsal, tail-like process; the other two, short, broad, triangular processes, one on each side of the body (Fig. 11). The macronucleus is roughly half as long as the body and lies in the anterior dorsal region. The left side of the body is hollowed out from in front of the middle progressively toward the rear.

We have found specimens which are identical with the typical *E. caudatum* except that the dorsal process is broad and short, instead of attenuated (Fig. 13). This form might be construed to be *E. bicarinatum* Cunha (1914). The only difference seems to be that of the size of the dorsal caudal process. Size, 81 x 40 $\mu$ , not including caudal processes.

We have found this species in more than one-third of the cattle we have examined.

17. *Entodinium bicarinatum* Cunha 1914

We discussed under *E. caudatum* what we believe might be this species. It resembles *E. caudatum* except that the dorsal spine is shorter, and the left side of the body is not as deeply hollowed out (Fig. 13). It may be a separate species or a young *E. caudatum*. Cunha found only a few isolated individuals. We have found a few in the material from each of six cattle. Size, 61 x 35 $\mu$ .

18. *Entodinium furca* Cunha 1914

This species is distinguished principally by two unequal caudal prolongations of the body, one dorsal, and one ventral (Fig. 12). Nuclei and contractile vacuole in dorsal region. Average size of our specimens, 52 x 27 $\mu$ .

We have encountered a few individuals of this species in several different cattle.

19. *Entodinium rostratum* Fiorentini 1889

The posterior end is rounded, and bears ventrally a caudal process which curves in slightly toward the axis of the body. Length of the spine is variable, but may be approximately as long as the body. Length of body, not including spine, 50 to 60 $\mu$ ; width, 20 to 30 $\mu$ .

We have not seen this species. We thought at first that we had found

it, but more careful scrutiny of the specimens showed a set of dorsal membranelles, thus eliminating them from the genus *Entodinium*.

## 20. *Entodinium dentatum* Stein 1859

There is no special distinctive feature about the anterior end of this species. The periphery of the posterior end is set with six incurved tooth-like prolongations of the body; two on each side, and one on each of the dorsal and ventral surfaces. Schuberg (1888) cast some doubt upon the propriety of placing this species in the genus *Entodinium*, for he says that a more exact study discloses that there is a second *Wimperzone*, and for such he created the genus *Diplodinium*. Eberlein (1895), however, again found *E. dentatum*, so that there can be little doubt of its legitimacy. Size, 60 to 90 $\mu$  by 30 to 50 $\mu$ . We have not seen this species.

### Genus *DIPLODINIUM* Schuberg 1888

The genus was created by Schuberg for a ciliate resembling *E. dentatum* Stein which possessed a second *Wimperzone*. He was probably wrong in assuming that Stein (1858) had overlooked a second zone of membranelles when he described *Entodinium dentatum*, for Eberlein (1895) later confirmed Stein's description. Stein gives no illustrations, however. It is not to be doubted as Sharp (1914) says, that Schuberg had before him a species different from *E. dentatum* Stein, and correctly referable to the genus *Diplodinium*, because it had a dorsal membranelle zone. Yet it must have been otherwise very much like *E. dentatum* Stein, and this species has six posterior tooth-like processes. *Diplodinium denticulatum* Fiorentini (1889) would resemble more the type species of the genus than *Diplodinium dentatum* Fiorentini (1889) which has only three posterior extensions of the body.

To state the matter briefly, the whole difficulty in accepting Sharp's suggestion to adopt *D. dentatum* as the type species is that Schuberg's *D. dentatum* is not *D. dentatum* Fiorentini, but *D. denticulatum* Fiorentini, which, as we shall explain below, is an entirely different species. We shall leave the matter for some future systematist to clear up.

## 21. *Diplodinium magii* Fiorentini 1889

This is the largest species of the protozoa found in cattle. The anterior surface is sharply truncated. There is an adoral zone of membranelles, and a dorsal zone which almost, but not quite, describes a complete circle or spiral as is figured by some authors. The membranelles are wanting in the ventral arc of the circle. The nucleus is shaped very much as in *D. bursa*. The general appearance is much the same as for *D. medium* except that the nucleus is typically pistol-shaped. The endoplasm is usually very dark and filled with a granular substance. With iodine a typical glycogen reaction is usually given. *Dasytricha* and *Entodinium* are often ingested as food, along with pieces of plant fiber. Movement is slow and cumbersome. We can sometimes see on the right side of the body a skeletal area resembling that of *D. bursa*. Does this species represent merely enlarged *D. bursa*, engorged with food and glycogen? It divides so as to produce two large individuals to be sure. We see no way of settling the question at present. Size, 175 to 255 $\mu$  by 120 to 175 $\mu$ .

We have found this species occasionally.

22. *Diplodinium bursa* Fiorentini 1899

This species is similar to *D. magii* in shape, being more "shaped like a heart with auricles cut squarely off" than *D. magii*. There is a collar-like ridge or fold of ectoplasm surrounding the operculum, the adoral and dorsal zones of membranelles (Figs. 21, 23). The anus at the posterior end is large and lined by a reflection of the cuticle. The two contractile vacuoles lie dorsal to the nucleus. Between the nucleus and contractile vacuoles is a bright line, (shown in black in figs. 21, 23), which probably represents a thickening of the cuticle. The macronucleus is pistol-shaped, and holds the one micronucleus in the bend between the handle and the barrel. It lies on the right side of the body.

There is one surface feature of this species which has been generally overlooked,—the skeletal. In this species it amounts to no more than a line of small irregular plaques passing longitudinally down the right side of the body (Fig. 21s). It forks at the anterior extremity. We regard the skeletal features as important in classification.

Size 100 to 150 $\mu$  by 60 to 90 $\mu$ . We find 140 by 85 $\mu$  to be the common size in our fixed material.

23. *Diplodinium medium* (Awerinzew and Mutafova 1914)  
Buisson 1923

Syn. *Metadinium medium* (Awer. and Mut. 1914)

This species is similar to *D. magii*, except that its macronucleus differs in shape. Instead of a pistol-shape, the nucleus presents an anterior, posterior, and median enlargement connected by narrower isthmuses. The micronucleus does not always lie in the angle just anterior to the median enlargement, but we have found it sometimes further anterior, close against the macronucleus. This species was found in but one cow, and could not be distinguished from the *D. magii* present there except by the character of the macronucleus (Fig. 24).

Size 187 to 270 $\mu$  by 136 to 175 $\mu$ .

24. *Diplodinium ecaudatum* forma *ecaudatum* Fiorentini 1889

This is the species which Sharp (1914) has immortalized by his inimitable work. We refer the reader to his work for the details and names of the various structures. There is no caudal spine in this variety (Fig. 22). Like the other members of this species, this variety has a skeletal area divided into right, left, and ventral regions, which meet about half way down the body, and pass posteriorly as a unit. We have found this variety frequently.

Size 112 to 140 $\mu$  by 40 to 60 $\mu$ .

*Diplodinium ecaudatum* forma *caudatum* Fiorentini

Syn. *Diplodinium caudatum* Fiorentini 1889

*Diplodinium rostratum* Fiorentini 1889

*Ophryoscolex inermis* Raillet 1890

*Ophryoscolex inermis* var. *caudatus* Cunha 1914

*Ophryoscolex intermixus* Awerinzew and Mutafova 1914  
nec. *Diplodinium caudatum* Eberlein 1895



Fiorentini (1890) himself suspected that his *D. ecaudatum* and *D. caudatum* were stages in the development of the same organism. Sharp (1914) has confirmed their identity, save for the inward curved spine which takes origin on the whole of the posterior end of the body which lies ventral to the anal opening (Fig. 25).

We find this species to be quite common in American cattle.

*Diplodinium ecaudatum* Fiorentini 1889 forma *bicaudatum* Sharp 1914

This species is exactly like the preceding, except for a smaller secondary spine situated just dorsal to the anal opening, and curving toward the primary spine.

We have found only two specimens of this variety, both from the same cow.

*Diplodinium ecaudatum* Fiorentini 1889 forma *tricaudatum* Sharp 1914

This variety is almost exactly like the preceding, except for a tertiary spine, which is located to the right of the median line (Fig. 25a). We have found specimens of this form in three different cattle.

*Diplodinium ecaudatum* Fiorentini 1889 forma *quadricaudatum* Sharp 1914

According to Sharp (1914), this variety is very similar to the preceding, except for the presence of a quaternary spine located on the right side of the body about midway between the primary and tertiary spines.

We have not seen this species. Sharp, who worked in California, states that he found it in the majority of the cattle that he examined, but we have not found it in Iowa.

*Diplodinium ecaudatum* forma *cattanei* Fiorentini 1889

Syn. *Diplodinium cattanei* Fiorentini 1889

*Ophryoscolex cattanei* Railliet 1890; da Cunha 1914

*Ophryoscolex fasciculus* Awerinzew and Mutafova 1914

This variety resembles all the others except that it possesses five posterior spines or prolongations of the body. There is always the larger ventral spine, as in forma *caudatum*, dorsal to which are the other four spines. We have found a few specimens of this variety in each of four cattle.

25. *Diplodinium eberleini* Cunha 1913

Syn. *D. caudatum* Eberlein 1895 (homonym)

*D. eberleini* Sharp 1914

This species is similar to *D. bursa*, according to Eberlein, except that the posterior end is drawn out into three processes. The dorsal process is figured almost as long as the body, and pointed. The two ventral processes, one on each of the right and left sides, are short, blunt and lip-like. Size 100 to 120 $\mu$  by 60 to 70 $\mu$ . We have not seen this species. Sharp reports that he did not find it in Pacific coast cattle.

26. *Diplodinium dentatum* Fiorentini 1889Syn. *D. mammosum* Railliet 1890*D. fiorentinii* Awerinzew and Metafowa, 1914nec. *D. dentatum* Railliet 1890; Eberlein 1895

This species is somewhat truncated at the anterior end. The macronucleus is elongated, somewhat bowed, and broader at the anterior end than at the posterior end (some exceptions). The micronucleus always lies in an indentation of the macronucleus at or just anterior to the middle of its dorsal surface. Fiorentini described three posterior tooth-like prolongations of the body. We often find specimens with two prolongations as figured (see Fig. 14), as well as those with three (Fig. 14a). Size 73 to 104 $\mu$  (not including spines) by 44 to 60 $\mu$ .

27. *Diplodinium denticulatum* Fiorentini 1889Syn. *Diplodinium dentatum* Railliet 1890; Eberlein 1895*Diplodinium dentatum* Fiorentini 1889 var.  
*denticulatum* Buisson 1923*Diplodinium anisacanthum* Cunha 1914

The validity of this species has been in dispute since Fiorentini described it. Among others, Eberlein (1895) believes it to be a form variation of *D. dentatum*. Buisson (1923) makes it a variety of *D. dentatum*. In the first place, however, the micronucleus of *D. denticulatum* has a constant position close to the macronucleus on its anterior dorsal convexity (Fig. 16). That of the two or three spined species, *D. dentatum*, is further posterior (Fig. 14). We believe that *D. anisacanthum* Cunha 1914, with a larger ventral spine, is merely an aberrant *D. denticulatum*, hardly deserving of even the rank of a variety, for we have found it among typical specimens. One often finds starch grains, plant fibers, and bacteria in the endoplasm of this species.

Size without spines, 54 x 43 $\mu$  to 82 x 55 $\mu$ .28. *Diplodinium minimum* sp. nov.*D. ecaudatum* Eberlein 1895

Eberlein identifies this form with *D. ecaudatum* Fiorentini. It is to be distinguished from *Entodinium minimum* only by the presence of a dorsal zone of membranelles, which is shaped exactly as in *D. bursa*. Posterior end is a somewhat rounded point. Size, 20 to 30 $\mu$  by 50 to 60 $\mu$ .

It can be seen at a glance that the size is quite different from that of *D. ecaudatum*, which Fiorentini gives as 120 by 44 $\mu$ . Another striking difference is the location of the dorsal zone of membranelles, which in *D. ecaudatum* is slightly posterior, and not on the dorsal edge of the anterior truncated surface. We have not seen this species.

29. *Diplodinium clevelandi* sp. nov.

We take great pleasure in naming this species after Doctor L. R. Cleveland, who showed by conclusive experimental evidence that the protozoa of the termite lived in symbiosis with their hosts.

This species is in general rectangular in outline, with adoral and dorsal zone of membranelles (Fig. 20). The macronucleus is elongated, and slightly curved in conformity with the dorsal margin of the body. The micronucleus lies in an indentation just anterior to the middle of the dorsal surface of the macronucleus. The anus opens externally in the indentation at about the middle of the posterior end of the body.

Another especially prominent feature of most specimens of this species is a v-shaped skeletal area on the right side, with apex away from adoral zone. There may be a less granular cycloid posteroventral extremity. There may be a very pronounced line between this clearer area and the thicker, more granular remaining portion of the body. Size  $112 \times 75\mu$  to  $78 \times 44\mu$ . Common size,  $93 \times 62\mu$ .

### 30. *Diplodinium hegneri* sp. nov.

At first we interpreted the smaller specimens belonging to this genus to be aberrant *D. ecaudatum*, and the larger ones either *D. bursa* or *D. magii*. The skeletal area on the right side of the medium sized individuals (ca  $120 \times 65$ ) is large and somewhat trapezoidal in outline (Fig. 17). Beneath the dorsal and ventral margins of the skeletal area are two refractile thickenings of the cuticle resembling attenuated rose thorns with the broader ends anterior, and the points continuous with the cuticle of the posterior limit of the body. In smaller specimens ( $82 \times 50\mu$ ) the skeletal area shows plainly, but the refractile cuticular thickenings are not pronounced. Many of the largest specimens ( $200 \times 100\mu$  and larger) resemble large engorged *D. bursa*, but here the character of the skeleton serves for distinguishing them. The cuticular supports have entirely or partially disappeared. All except the dorsal and ventral portions of the skeletal area has become so thin, that the only remaining evidence for a skeletal area is two parallel diagonal splinters of skeleton on the anterior half of the right surface of the animal. These stain deep brown to violet with iodine when the material is fresh and unfixed. Contractile vacuoles are dorsal and number two in the smaller specimens, and up to five or more in the larger ones. Transverse binary fission may take place at any stage of development.

The developmental cycle through which this species passes can be followed continuously from the smaller specimens up to the largest, and we have no doubt that all the different stages belong to one species. The case may be similar to that of *D. magii* and *D. bursa*, where it is entirely possible (we think probable) that *D. magii* represents an enormously enlarged *D. bursa*, and the fact that both may have an almost identical skeletal area lends additional evidence.

We take great pleasure in naming this species after Doctor Robert W. Hegner of Johns Hopkins University under whose stimulating influence the senior author was initiated into the field of protozoology.

### 31. *Diplodinium helseri* sp. nov.

This species is a typical *Diplodinium* with adoral and dorsal zone of membranelles, the latter not strikingly conspicuous, but nevertheless always present (Fig. 19). There is a caudal ventral prolongation of the body, often very much incurved. The macronucleus is elongated, blunt at anterior end and somewhat pointed at posterior end. The micronucleus lies in a dorsal

indentation of the macronucleus. The protoplasm is granular, but not densely so. The posterior-dorsal portion of the body is somewhat thinner and more transparent than the remaining portions of the body. Length of body, not including spine, 43 to 58 $\mu$ ; width 25 to 33 $\mu$ . Length of spine 13 to 25 $\mu$ .

This species is fairly frequently found in cattle.

We have named this species after Professor M. D. Helser in appreciation of the co-operation he has lent us.

Genus *OPHRYOSCOLEX* Stein 1858

Body more or less ovoid, truncated at the anterior end. Posterior end either rounded or bearing a number of elaborate spine-like prolongations of the body. Mouth surrounded by adoral zone of membranelles. Dorsal zone of membranelles surrounds four-fifths of the circumference of a zone somewhat anterior to the middle of the body. Contractile vacuoles are numerous, five, six or more. We are not convinced that the three species of *Ophryoscolex* which have been described are not in reality all one species. Until they have been cultivated in pure lines, however, one would not be justified in uniting them into a single species.

32. *Ophryoscolex inermis* Stein 1858  
nec. *O. inermis* Railliet 1890

Agrees in general with the generic description, except that the posterior end is rounded, and not provided with spines. Size, 170 to 190 $\mu$  by 65 to 100 $\mu$ .

We have found a few specimens of this genus in the one cow in which we found *O. caudatus* in large numbers. It may represent merely a fluctuation of the latter species.

33. *Ophryoscolex purkynjei* Stein 1858  
Syn. *Diplodinium vortex* Fiorentini 1889

This species agrees with the preceding one, except that the posterior end is not rounded. Instead it is bristling with thorn-like appendages, or prolongations of the body. These are arranged in two or three terraces, each terrace encircling the body except for a short arc on the ventral surface. At the posterior extremity is a bifid spine.

Size, 200 by 80 $\mu$ .

We have not found this species.

34. *Ophryoscolex caudatus* Eberlein 1895

This species is like the preceding, except that the body terminates in one long spine (Fig. 26).

We have not found individuals with such long spines as Eberlein figures. Those we find have one broad spine on the posterior tip, somewhat larger than the other spines in the posterior region. We think ours belong to this species, however. Fig. 27 shows the skeletal area which is mostly ventral. It reminds one very much of that structure in *D. ecaudatum*,

which is tripartite anteriorly, but the parts blend for a considerable distance, only to again become tripartite posteriorly.

#### CULTIVATION EXPERIMENTS

Our first attempt at cultivation of the cattle protozoa was made on Feb. 16, 1926. The protozoa were obtained at the abattoir from cow 5, and carried to our laboratory in a thermo-jug. Tubes of media were inoculated immediately and incubated at 35°C. Three different kinds of media were used.—Tanabe's, Locke-egg-albumen, and Hogue's ovomucoid. Tanabe's medium was made from .7 gram NaCl, 1 gram sodium citrate, .5 gram Löffler's blood serum, 2 c.c. white of egg and 100 c.c. distilled water. For the Locke-egg-albumen medium, egg slants were made from four eggs shaken with 50 c.c. of Locke's physiological solution; a solution of 8 parts Locke's solution (distilled water, 1,000 c.c.; NaCl, 9 grams; CaCl<sub>2</sub>, .2 grams; KCl, .4 grams; NaHCO<sub>3</sub>, .2 grams; and glucose, 2.5 grams), and one part crystallized egg albumen was added to the depth of one cm. above the egg slant. The Hogue ovomucoid medium, was made from the whites of six eggs shaken with 600 c.c. of .7 per cent NaCl solution. The mixture was cooked over a boiling water bath for 20 minutes and strained through cheese cloth. It was tubed and autoclaved for 20 minutes at 15 pounds pressure.

After 45 hours, the Tanabe's medium showed no signs of growth; both the ovomucoid and the egg slants contained a few active *Isotrichs*. Subcultures were made from all three kinds of media. Twenty-four hours later, the *Isotrichs* were all dead and there seemed to be no life in any of the tubes. However, after another 24 hours, the ovomucoid medium was filled with small, active amoeba. We identified them as *Vahlkampfia lobospinosa* except that they lacked a contractile vacuole. Subcultures were made every other day and the amoebae were kept until April 2. Apparently the medium ceased to supply the nutritional requirements after 45 days.

More tubes of the ovomucoid medium were inoculated on February 23 from cow 7. The ciliates did not live for more than 48 hours. *Trichomonas bovis*, however, did live in this medium and was cultivated for three weeks by subculturing every other day.

Later in the year we tried various different kinds of media. The incubator was set at 37½° C. instead of 35°. Both aerobic and anaerobic technique was used in all cases. A mixture of Loeffler's blood serum, .5 grams; sodium citrate, 1 gram; and 0.5 per cent salt solution, 100 c.c. was used alone and mixed with corn meal and alfalfa. Corn and alfalfa were used in 0.5 per cent salt solution. Alfalfa infusion was used in 0.8 per cent solution and in distilled water. Alfalfa was used with ovomucoid medium. Inactivated human blood serum was mixed with salt solution in the proportion of 6 to 100 c.c. None of these different media were successful for cultivating cattle protozoa.



TABLE 1. INCIDENCE OF VARIOUS SPECIES OF CILIATES IN CATTLE

[illegible]

## GENERAL DISCUSSION

The survey shows that with the exception of the three species of *Bütschlia* we may expect to find about the same general types of protozoa in American cattle as are found in the Old World. We have found three new species of ciliates, which may have been either acquired from some other ruminant indigenous to North America, as the bison, or overlooked in European cattle. Also, a cow may have as few as two species of ciliates in its stomach, or as many as sixteen. It would be difficult to draw any definite conclusions as to the associations of any definite groups of species, as an inspection of the accompanying table will show.

There are several interesting observations regarding life histories which should be emphasized in passing. *Diplodinium hegneri* presents a variety of stages in its life cycle. A series can be constructed of forms ranging from small up to extremely large, with marked differences of skeletal structure at the extremes of the series; nevertheless, a convincing series of intermediate stages can be found. Then, there are certain reasons why *D. magii* may be considered to be an enlarged *D. bursa*. Their shape and general structure are similar. But more convincing is the fact that both possess the same type of skeletal area, a small horseshoe-nail-shaped area on the right side.

From the standpoint of evolution, there is plenty of food for thought in the complex ciliates found in cattle. Professor C. A. Kofoed was quoted in the newspapers several years ago as having mentioned high-speed evolution among termite protozoa. We might just as appropriately speak of high speed evolution among the protozoa of ruminants. We have various species of *Entodinium* with only an adoral zone of membranelles. Then the dorsal zone of membranelles on which the genus *Diplodinium* is established appears. In this genus is the apparently unstable species, *Diplodinium ecaudatum*, with a dorsal zone of membranelles more posteriorly located than in other members of the genus, a tripartite skeletal area (at least anteriorly), and a tendency to vary in regard to the number of caudal spines. Apparently, from this species *Ophryoscolex* was derived. It has the dorsal zone of membranelles much further removed from the anterior end, a skeletal area suggesting that of *D. ecaudatum* in that it is tripartite anteriorly, and a tendency to form a very elaborate armament of posterior spines.

What is the rôle of these protozoa in the stomach of the ox? We shall not discuss in detail the various suggestions that have been advanced, for it is still an unsolved problem. Among the views that have been put forth are these: (1) they assist in the digestion of the food of the cow, (2) they are injurious parasites of the cow, (3) they serve to reduce the mould population of the rumen by feeding upon it, (4) they assist in digesting cellulose, (5) they supply a meat diet to the cow by multiplying in great numbers and passing with the food mass to the omasum and abomasum where they are digested, (6) they are mere commensals. Concerning the true significance, however, of this flourishing population of micro-organic animal life in the stomach of the cow, we can at present only conjecture. Carefully planned research should serve to determine which of the six aforementioned hypotheses can be established on facts.

## KEY TO PROTOZOA INHABITING RUMEN AND RETICULUM OF CATTLE

1. Locomotion by means of pseudopod formation (3).
2. Locomotion by means of flagella or cilia (5).
3. Nucleus with conspicuous karyosome—*Vahlkampfia lobospinosa* (Fig. 1).
4. Nucleus with inconspicuous punctiform karyosome—*Endamoeba bovis*.
5. Locomotion by means of flagella (7).
6. Locomotion by means of cilia or membranelles (15).
7. Not possessing axostyle (9).
8. Possessing axostyle (13).
9. Possessing one long flagellum (11).
10. Possessing about twelve united lateral flagella—*Callimastix frontalis* (Fig. 3).
11. Spherical—*Monas communis*.
12. Pear-shaped, flagellum arising laterally—*Piromonas communis*.
13. With undulating membrane—*Trichomonas communis* (Fig. 5).
14. Without undulating membrane—*Eutrichomastix ruminantium* (Fig. 4).
15. Locomotion by means of cilia covering body (17).
16. Locomotion by means of membranelles in anterior region. No cilia on other portions of body (27).
17. Cilia conspicuous all over body (19).
18. Cilia conspicuous in circumoral region at anterior end, inconspicuous over other portions (23).
19. Rows of cilia (striations) longitudinal (21).
20. Rows of cilia (striations) spiral—*Dasytricha ruminantium* (Fig. 8).
21. Mouth-like opening at posterior end—*Isotricha prostoma* (Fig. 6).
22. Mouth-like opening definitely posteriolateral—*Isotricha intestinalis* (Fig. 7).
23. Lanceolate, collar-like anterior constriction—*Bütschlia lanceolata*.
24. Not elongated; no collar-like constriction, anterior end flattened (25).
25. Posterior end rounded—*Bütschlia parva* (Fig. 9).
26. Posterior end somewhat pointed, provided with four posterior-lateral indentations—*Bütschlia neglecta*.
27. Adoral zone of membranelles only (29).
28. A dorsal zone of membranelles in addition to adoral zone (41).
29. Without caudal spine-like prolongations of body (31).
30. With one or more caudal spine-like prolongations of body (33).
31. Macronucleus about four-fifths body length—*Entodinium bursa* (Fig. 10).
32. Macronucleus much shorter; smaller forms—*Entodinium minimum* (Fig. 15).

33. One long ventral caudal process—*Entodinium rostratum*.
34. More than one caudal processes (35).
35. One dorsal and one ventral process—*Entodinium furca* (Fig. 12).
36. Three or more caudal processes (37).
37. Six tooth-like caudal processes—*Entodinium dentatum*.
38. Three caudal processes (39).
39. Dorsal caudal process several times as long as two ventral caudal processes—*Entodinium caudatum* (Fig. 11).
40. Dorsal caudal process about same length as two ventral caudal processes—*Entodinium bicarinatum* (Fig. 13).
41. Dorsal zone of membranelles four-fifths encircling body just anterior to middle (43).
42. Dorsal zone of membranelles not so extensive, at anterior end (47).
43. Posterior end rounded; no spines—*Ophryoscolex inermis*.
44. Posterior end bristling with spines (45).
45. Most posterior spine bifid—*Ophryoscolex purkynjei*.
46. Most posterior spine elongated, not bifid—*Ophryoscolex caudatus* (Figs. 26, 27).
47. Surface skeletal area with anterior right, left, and ventral divisions meeting about middle of body. Length 112 to 140 $\mu$ —*Diplodinium ecaudatum*.
  - (a) no caudal spine, forma *ecaudatum* (Fig. 22).
  - (b) one caudal spine, forma *caudatum* (Fig. 25).
  - (c) two caudal spines, forma *bicaudatum*.
  - (d) three caudal spines, forma *tricaudatum* (Fig. 25a).
  - (e) four caudal spines, forma *quadricaudatum*.
  - (f) five caudal spines, forma *cattanei*.
48. Otherwise (49).
49. Skeletal area on right side of body only (51).
50. Skeletal area inconspicuous or not present (53).
51. Skeletal area extensive, extending to posterior end of body—*Diplodinium hegneri* (Fig. 17).
52. Skeletal area splinter-like slab only—*Diplodinium bursa* (Fig. 21).  
(See also *D. magii*).
53. Giant forms, 175 to 275 in length (55).
54. Smaller forms (57).
55. Macronucleus pistol-shaped—*Diplodinium magii*.
56. Macronucleus yoke shaped—*Diplodinium medium* (Fig. 24).
57. With one or more caudal prolongations of body (59).
58. Without caudal prolongations of body (65).
59. One incurved caudal process; small forms—*Diplodinium helseri* (Fig. 19).
60. More than one caudal process (61).

61. With six (or five) tooth-like caudal spines—*Diplodinium denticulatum* (Fig. 16).
62. With three (or two) caudal processes (63).
63. Dorsal spine almost as long as the body, pointed; two lateral ventral flap-like processes—*Diplodinium eberleini*.
64. Three (or two) posterior tooth-like processes—*Diplodinium dentatum* (Figs. 14, 14a).
65. Body somewhat rectangular, posterior end rounded—*Diplodinium clevelandi* (Fig. 20).
66. Body somewhat elongated, triangular, tapering to posterior end—*Diplodinium minimum*.

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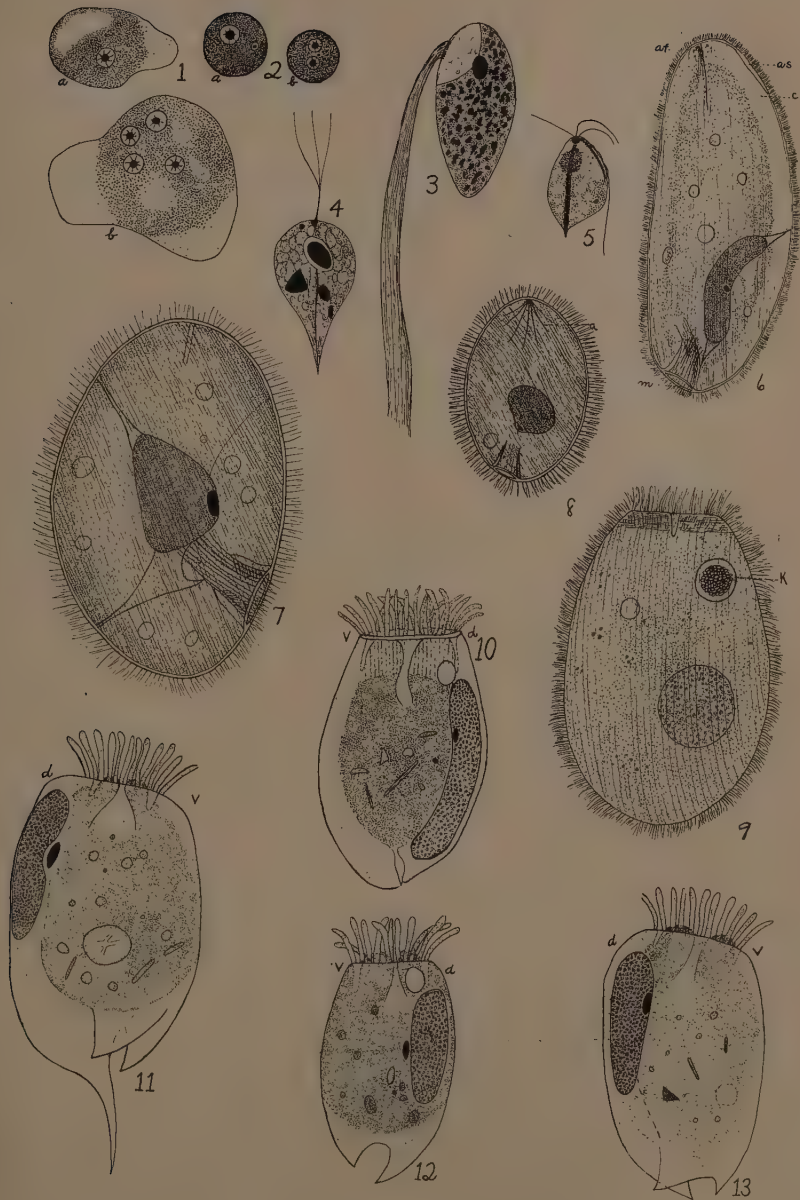
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## EXPLANATION OF PLATES

## PLATE I.

- Fig. 1. *Vahlkampfia lobospinosa*, trophozoites from cultures. (a) uninucleated form (b) large individual with four nuclei. X ca. 1240.
- Fig. 2. *Vahlkampfia lobospinosa*, cysts. (a) uninucleated cyst, with small chromatoid; (b) binucleated cyst. X ca. 1240.
- Fig. 3. *Callimastix frontalis*, trophozoite (after Braune). Lighter anterior area is Stirnfeld, with basal granules of flagella in margin. X ca. 2100.
- Fig. 4. *Eutrichomastix ruminantium*, trophozoite (after Braune). X ca. 2100.
- Fig. 5. *Trichomonas ruminantium*, trophozoite from culture. X ca. 1775.
- Fig. 6. *Isotricha prostoma*, trophozoite. af, Braune's *Afterfibrillen*; as, Stein's and Schuberg's *Afterspalte*; c, cortical ectoderm; m, "mouth" at posterior end. X 375.
- Fig. 7. *Isotricha intestinalis*, trophozoite. X 750.
- Fig. 8. *Dasytricha ruminantium*, trophozoite. a, Braune's *Afterstützen*. "Mouth" is opening at posterior end. X ca 490.
- Fig. 9. *Bütschlia parva*, trophozoite (after Schuberg, modified). X ca. 1280. k, *Concremenhaufen*.
- Fig. 10. *Entodinium bursa*, trophozoite. d, dorsal side; v, ventral. X 750.
- Fig. 11. *Entodinium caudatum*, trophozoite. d, dorsal side; v, ventral side. X 750.
- Fig. 12. *Entodinium furca*, trophozoite. X 750.
- Fig. 13. *Entodinium bicarinatum*, trophozoite. X 750.



## PLATE II.

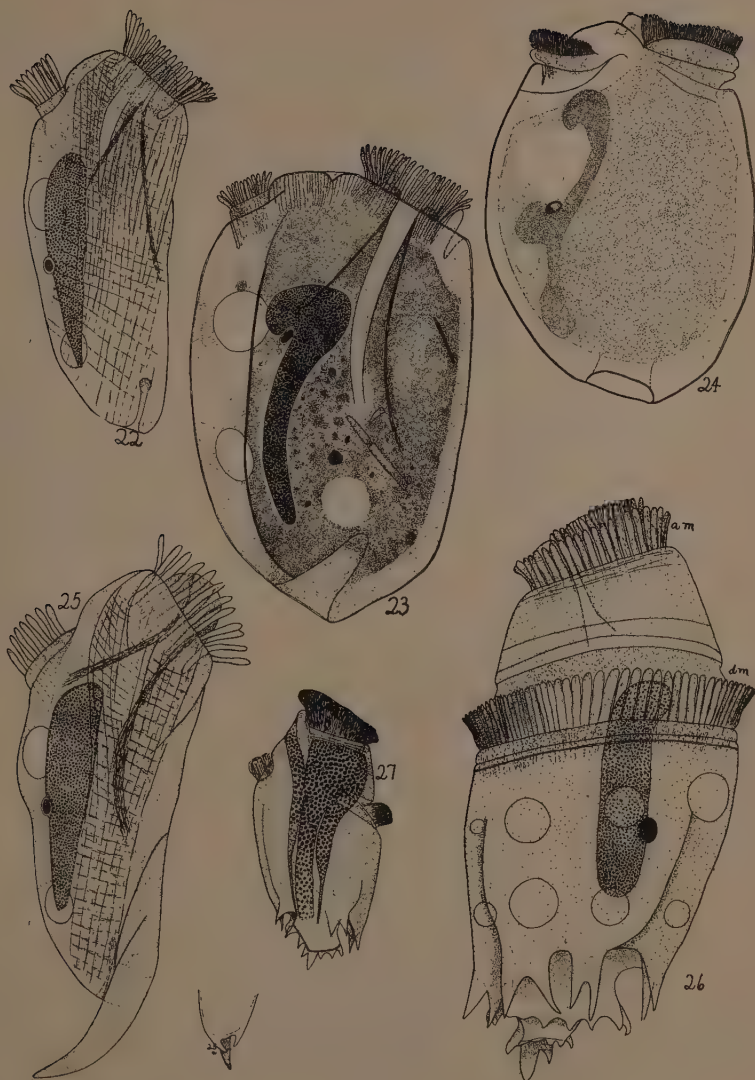
- Fig. 14. *Diplodinium dentatum*, trophozoite with two posterior processes. X 750. 14a, posterior end of specimen with three processes, the more typical condition. X 375.
- Fig. 15. *Entodinium minimum*, trophozoite. X 750.
- Fig. 16. *Diplodinium denticulatum*, trophozoite. X 750.
- Fig. 17. *Diplodinium hegneri*, medium-sized trophozoite. am, adoral zone of membranelles; dm, dorsal zone of membranelles; op, operculum; s, skeletal area on right side; c, the cuticular supports of skeleton. X 750.
- Fig. 18. *Diplodinium hegneri*, large trophozoite, from left side. f, oesophageal retractor fibers. X 375.
- Fig. 19. *Diplodinium helseri*, trophozoite. X 750.
- Fig. 20. *Diplodinium clevelandi*, trophozoite. am, adoral zone of membranelles; dm, dorsal zone of membranelles. X 375.
- Fig. 21. *Diplodinium bursa*, right side view, showing skeletal area, s. This is similar to skeletal area to be seen on right side of many specimens of *D. magii*. X 375.





## PLATE III.

- Fig. 22. *Diplodinium ecaudatum* forma *ecaudatum*, showing left and ventral skeletal areas. X 500.
- Fig. 23. *Diplodinium bursa*. X 500.
- Fig. 24. *Diplodinium medium*. Note characteristic macronucleus. X 250.
- Fig. 25. *Diplodinium ecaudatum* forma *caudatum*. X 500. 25a, posterior end of forma *tricaudatum*. X 250.
- Fig. 26. *Ophryoscolex caudatus*, dorsal view. am, adoral zone of membranelles; dm, dorsal zone of membranelles. X 500.
- Fig. 27. *Ophryoscolex caudatus*, ventral view showing skeletal areas. X 250.





## CHEMICAL TESTING OF NICOTINE DUSTS\*

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The introduction of nicotine as a dusting agent has induced several laboratories to develop chemical methods for testing the efficiency of different dusts as carriers. The most extensive work has been that of Thatcher and Streeter (1923). Literature citations will be found in these publications.

In testing several dusts with the object of finding the most economic carrier for the work in Iowa, the method of Thatcher and Streeter was selected as the most convenient. In applying this method to bentonite dust, irregularities were encountered which could not be explained as due to experimental error. In checking up this abnormality for bentonite, it developed that the method of Thatcher and Streeter is not a measure of the efficiency of the carrier but gives rather a measure of the rate of evolution of nicotine vapor plus the rate of decomposition of the nicotine.

### LOSS OF NICOTINE DURING STORAGE

Thatcher and Streeter pointed out that certain carriers, especially calcium carbonate, suffered considerable loss of nicotine even when stored in sealed containers. They suggested that this might be due to some chemical change in the nicotine. In a special study for "the loss of nicotine from nicotine dusts during storage," McDonnell and Young confirm this observation.

In working with bentonite as a carrier, it was found that 23% to 86% of the nicotine added was unaccountably lost when the dust was allowed to stand in tightly stoppered bottles for 24 to 48 hours. This loss took place independently of whether the nicotine was added as the sulfate after treating with lime or as the free nicotine.

Later experiments using pure nicotine revealed that an ether soluble oil was left in the bentonite after exposure; the insolubility of this oil in water and dilute acids would indicate that it might be oxy-nicotine.†

### DETERMINATION OF NICOTINE VAPOR FROM VARIOUS DUSTS

Since the primary object of the laboratory work was the finding of an economic carrier, the nature of the change in the nicotine was not investigated but attention was turned to the design of an apparatus that would permit the analysis of the vapor given off by the various dusts.

\* *Note.* The data on which this report is based have been accumulated over a period of two years, attention being limited to practical aspects of the question. In view of the contradictory nature of our evidence in regard to that in the literature, it seemed advisable to publish this incomplete report. The theoretical questions are now being studied and will be reported at a future date. The analytical work has been carried out by W. Catlin, M. B. Strawn and E. Winton.

† Experimental work by W. R. Harlan.

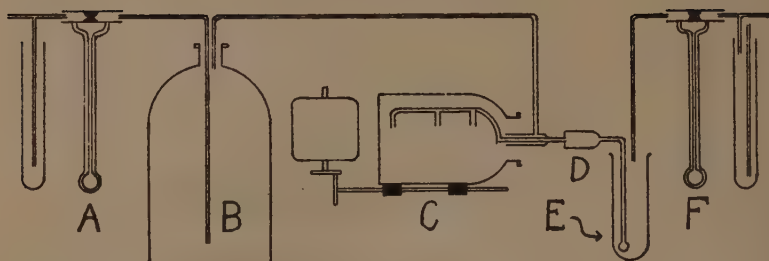


Figure 1

The apparatus shown in Fig. 1 was designed to simulate actual dusting conditions. By means of flowmeter A, air at a definite rate is passed through the humidity control B and allowed to blow over the surface of any dust agitated in the mixing machine\* C.

The dust is contained in a rotating bottle so that a film of dust is continuously falling past the sampling tube leading to the filter D. The sample of air-nicotine mixture above the dust is drawn by suction thru flowmeter F at the same rate as the air enters the mixing bottle, the dust is removed

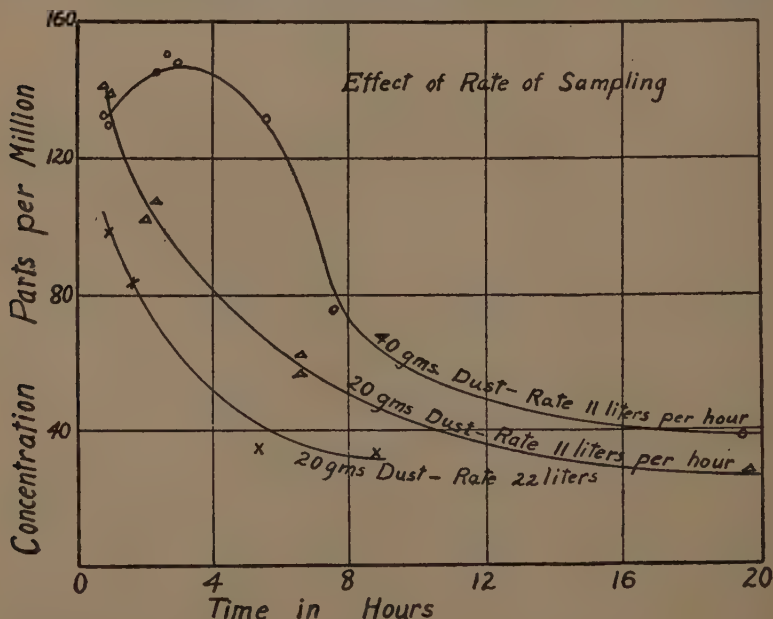


Figure 2

\* The construction of this machine is described by Hixon in the JI. Ind. & Engr. Chem. (1926) 18, 138.



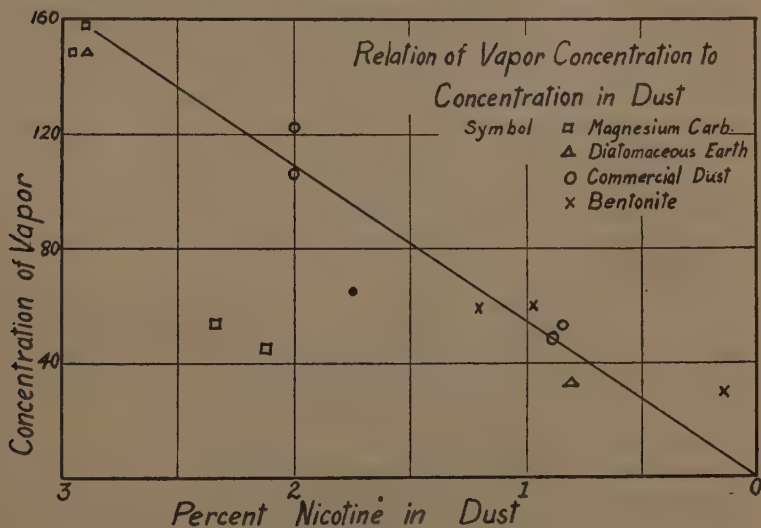
by cotton in filter D and the nicotine absorbed in the train E. It is possible, by this apparatus, to determine the amount of nicotine left in the powder after exposures of various lengths of time.

It was expected that equilibrium conditions could be established similar to those in use in determining vapor pressures by the dynamic method. This has not proved to be the case; Fig. 2 shows typical curves obtained under various rates of sampling. So far we have not attempted to control the light factor nor the speed of agitation of the dust. Without controlling these factors, checks within about 10% are obtained on duplication of all conditions.

In Table I, results are reported for the analysis of 3% nicotine on bentonite, diatomaceous earth and magnesium carbonate as carriers and a 2% commercial dust. It will be seen that from 7% to 91% of the nicotine is not accounted for depending upon the carrier; and that the concentration of the nicotine in the vapor is in the inverse order varying from 150 to 60 parts per million.

TABLE I.  
VAPOR CONCENTRATION AND NICOTINE LOSS FOR FOUR DUSTS

| Kind of dust           | Hour of analysis | Concentration of vapor in parts per million | Vapor removed in air | Remaining in dust | Not accounted for |
|------------------------|------------------|---|----------------------|-------------------|-------------------|
| 3% Bentonite           | 0-2              | 61  |                      |                   |                   |
|                        | 14-17            | 31  | 8.6%                 | 0.1%              | 91%               |
| 2% Commercial Dust     | 0-1              | 110   |                      |                   |                   |
|                        | 14-17            | 50  | 10%                  | 45%               | 45%               |
| 3% Diatomaceous Earth  | 0-1              | 141   |                      |                   |                   |
|                        | 10-12            | 36  | 10%                  | 25%               | 65%               |
| 3% Magnesium Carbonate | 0-1              | 150   |                      |                   |                   |
|                        | 15-17            | 50  | 13%                  | 80%               | 7%                |



In Fig. 3, the concentration of the vapor has been plotted against the nicotine content of the dust as revealed by analysis. It will be observed that with the exception of magnesium carbonate dust, the vapor concentration is roughly proportional to the nicotine content of the dust. Magnesium carbonate dust rapidly forms small balls which prevent uniform contact with the air, thereby lowering the rate of volatilization and decreasing the rate at which the nicotine is decomposed.

All of this evidence indicates that the nicotine is decomposed to a varying degree depending upon the catalytic nature of the dust. In view of the chemistry of nicotine, the most logical explanation seems to be that the nicotine is oxidized by the air to a non-volatile form. This would also account for the failure to obtain a constant concentration of nicotine vapor in the above apparatus as would be expected, since varying degrees of decomposition of the nicotine in the gas phase would take place according to the velocity of sampling.

#### TOXICITY OF NICOTINE

The toxicity of nicotine has been studied by McIndoo (1916), who concludes that its killing properties are due to paralysis. Headlee reports the concentration of nicotine vapor in a field dusted under practical conditions. Calculation of his results show from 8-15 parts of nicotine per million parts of air.

It was expected that the toxicity of nicotine vapor could be easily studied in the apparatus shown in Fig. 1 by connecting vessels containing insects between the filter D and the absorption bottles E.

Preliminary experiments with the cabbage aphid, showed that when these insects still feeding on cabbage leaves were placed in a 250 cc. bottle and connected to the apparatus, a concentration of 75-100 parts nicotine per million of air caused complete dropping of the insects in two minutes' time. Since the velocity with which the sample was drawn through the bottle was 175 cc. / min. this period of time would be required to displace the air from the bottle containing the insects. These insects were exposed for another six minutes and then transferred to bottles free from nicotine, the insects showed complete recovery in 12 hours.

Since the aphid is too susceptible to nicotine for experimental purposes, the very resistant rice weevil (*Sitophilus oryza* L.) was used for experimentation on toxicity. Preliminary experiments show that from 160 to 200 parts nicotine per million of air will give from 60 to 100% kill on the weevil when exposed for three hours.

Biological proof of the decomposition of nicotine in the vapor state can be seen in the following experiment. Nicotine vapor at a concentration of 160 pts. /mil. was drawn through a series of three bottles containing weevils. The first bottle was removed after 3 hours' exposure, the second and third bottles being exposed for 2 hours longer. Counts were made after 48 hours and the kill found to be 60%, 50%, 25%, respectively showing that although the last two bottles were exposed for a longer period of time the toxicity of the nicotine was less than that in the first bottle. In view of the apparent rapidity of the decomposition of nicotine vapor in the air, the data given here as well as that in other portions of the paper should be considered as of comparative value rather than of a precise nature.

This toxicity of nicotine is greater than that of hydrogen cyanide. The

mere fact that one of the Coleoptera is so susceptible to nicotine is rather contradictory to present conclusions concerning dusting.

Another very significant fact is that if small quantities of dust pass the filter and come in contact with the insects a much more rapid and effective kill is obtained. Whether this implies that nicotine is more effective as a contact poison or merely that the concentration of the vapor is much higher at the surface of the dust remains to be proven.

### SUMMARY

1. It is pointed out that the method proposed by Thatcher and Streeter for chemical testing of the efficiency of various carriers for nicotine dusts is not reliable.

2. An apparatus is described for analyzing the nicotine vapor evolved from a dust and results reported on the toxicity of this vapor to insects.

3. The evidence indicates that nicotine decomposed at various rates in the dust is presumedly due to oxidation.

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## EFFECT OF CONCENTRATION AND TEMPERATURE ON GERMICIDAL EFFICIENCY OF SODIUM HYDROXIDE\*

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Mechanical washing of bottles is being universally adopted in the beverage and milk industries. Numerous washing compounds are now available on the market for use in conjunction with these washing machines, but there is a dearth of information as to their relative efficiencies either as cleansing agents or germicides. These washing compounds consist of caustic soda, sodium carbonate, trisodium phosphate, sodium silicate or various mixtures of these alkalies. The mixtures most commonly encountered consist of caustic soda as a base to which are added various quantities of sodium carbonate or trisodium phosphate.

The sanitary regulations of many states and cities specify the concentration of alkali to be employed calculated on the basis of sodium hydroxide as determined by titration with acid. The question at once arises whether a sodium carbonate or phosphate solution which titrates 2% alkali, calculated as sodium hydroxide, is as efficient as 2% caustic soda. What is the effect of temperature on the germicidal properties of alkalies, and how is the disinfecting power of caustic soda affected by the additions of the carbonates and phosphates? Such information is essential for the proper and efficient regulation of bottle cleansing and sterilization.

In this paper are recorded some observations on the effects of concentration and temperature on the germicidal efficiency of sodium hydroxide. Additional reports will deal with other alkalies, the effect of addition of various salts on the disinfecting powers of sodium hydroxide, and the influence of  $H^+$  ion concentration.

Sodium hydroxide was employed as a basis for comparison in these studies because it constitutes the foundation of most of what are generally considered the better washing compounds on the market. As the concentrations of alkali and temperatures generally recommended for mechanical washing are quite high, a resistant organism was selected for comparative studies. A technique had to be developed which would permit of rapid cooling and neutralization of the alkali, conform in a general way to conditions encountered in washing machines, and not introduce too great an error in bacterial counts.

*Characteristics of organism employed.* The organism employed was isolated from a sample of spoiled ginger ale. It was a gram positive rod (about  $1.0\mu$  by  $2.0$  to  $4.0\mu$ ), facultative, motile, with central spores equal to or slightly less than the diameter of the cells. The vegetative cells occurred singly, in pairs, and occasionally in short chains. Gelatin was liquefied, milk slowly curdled (rennet), and peptonized, nitrates were reduced

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\* The work here reported was made possible through a grant from the American Bottlers of Carbonated Beverages.



to nitrites but not gas, and indol was formed. Acid was formed from glucose but not from lactose nor sucrose and starch was hydrolyzed. Colonies on agar were strikingly similar to those of *B. subtilis*.

The organism resembles *Bacillus laterosporus* and *B. ruminatus* as described by Bergey. It differs from the former in that nitrates are reduced to nitrites and starch is hydrolyzed, and it deviates from the latter in reducing nitrates, but failing to ferment sucrose.

The organism grew very rapidly on nutrient agar, forming distinct but not too large colonies in 2 days and as there was very little tendency of the colonies to confluence the culture was well adapted for plate counts.

*Preparation of test culture suspension.* A 24 hour broth culture was smeared over the surfaces of nutrient agar (Difco) in 9 Kolle flasks and incubated at 25-27°C. for 19 days after which period practically all of the organisms appeared to be in the spore stage. The surface growth was then scraped off into a sterile evaporating dish and dried over sulfuric acid in a partial vacuum. The dried mass was then ground to a fine powder in an agate mortar and thoroughly mixed with sterile powdered cane sugar. The mixture was placed in a sterile weighing bottle and kept in a desiccator over sulfuric acid. From time to time small quantities of the spore sugar mixture were removed and the number of viable organisms per unit weight ascertained.

*Technique of disinfection tests.* Approximately 0.02 to 0.05 gram of the bacterial suspension was placed in 10 cc. of sterile tap water and after thorough shaking the suspension was filtered through a fine grade of filter paper to remove clumps. The apparently homogeneous filtrate constituted the bacterial suspension employed for disinfection.

The test alkali (100 cc.) was placed in a 200 cc. round-bottom Woulff bottle, provided with three necks or openings. A glass stirrer was inserted through a stopper in the middle neck; the other openings were stoppered with cotton and the entire apparatus sterilized in the autoclave at 120°C for 15 to 20 minutes. After cooling, the flask containing the alkali was placed in a DeKhotinsky water bath and the stirrer set in motion, taking care that no bubbles or foaming developed.

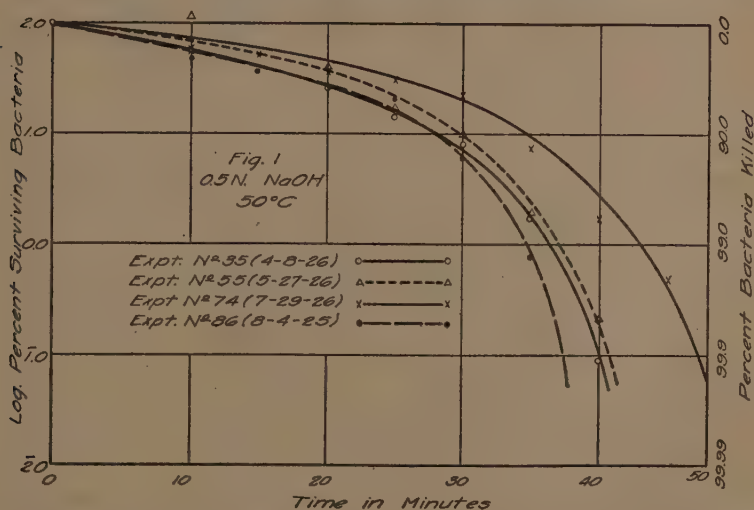
When the alkali attained and remained at the desired temperature, 1.0 cc. of the bacterial suspension was introduced under the surface of the liquid by means of a capillary pipette. At desired time intervals, 5.0 cc. portions of the thoroughly stirred mixture were removed and inoculated into Erlenmeyer flasks containing 45 cc. of sulfuric acid (with methyl orange indicator) of a strength just sufficient to neutralize the alkali added. The effects of alkali and temperature were thus simultaneously stopped. The number of surviving bacteria was then determined by plating on nutrient agar. (To reduce variations introduced by using different batches of culture media, a single batch of Difco dehydrated nutrient agar was employed for all the experiments). All bacterial counts were calculated on the basis of 5.0 cc. of disinfecting mixture. It was aimed to employ a suspension giving an initial count of about 1,000,000 per unit volume (5.0 cc.).

#### 1. Effect of Concentration of Sodium Hydroxide on Killing Time at 50°C

Concentrations of 0.5N, 0.75N, 1.0N and 1.25N sodium hydroxide were employed at 50°C. With the technique employed, each colony developing on nutrient agar to which had been added 1.0 cc. of the cooled and neutral-

ized alkali from the Erlenmeyer flask was equivalent to 50 bacteria per unit disinfecting volume (5.0 cc.). It is generally conceded that bacterial counts based on less than 15 to 20 colonies per plate are subject to considerable error and are therefore unreliable so that bacterial counts of less than 700 to 1,000 could not be depended upon. With an initial count of 1,000,000 reductions up to about 99.9% can be readily and reliably ascertained, but estimates of further reductions are subject to considerable error due to the small number of colonies developing per plate. In this study comparisons are based on the time required for effecting a reduction of 99.9% of the exposed organisms.

a. *Experiments with 0.5 Normal Sodium Hydroxide.*—In Fig. 1 four of the individual experiments showing the extreme variations are plotted



and in Fig. 10 the average for the ten series of 0.5N. sodium hydroxide at 50°C. is shown. In order to facilitate comparisons, the logarithms of the per cent surviving bacteria are plotted against the time, thus giving a common initial point for all curves. In Table I are shown the numbers of surviving bacteria, as well as the average per cent survivors and their logarithms.

It will be observed from Table I that although determinations were made on different occasions between April 6 and September 3, the killing time for 99.9% of the exposed organisms was quite constant, namely 38 to 42 minutes in nine of the ten experiments.

The similarity in the course of disinfection in each of the experiments is very striking. From the curves in Fig. 1 it will be seen about 40% of the bacteria were killed in the first ten minutes, the rate of death per cell was slightly greater during the next ten minutes, but thereafter the rate increased progressively with time. Thus, of the number of organisms present at the beginning of each interval, approximately 47% were killed dur-

ing the first, 45% in the second, 76% in the third and 96% in the fourth 10 minute period.

TABLE I  
SHOWING SURVIVING BACTERIA IN 0.5N SODIUM HYDROXIDE AT 50°C.

| Expt. No.<br>Date, 1926 | 27<br>4/6                      | 35<br>4/8 | 49<br>5/22 | 55<br>5/27 | 74<br>7/29 | 78<br>8/4 |
|-------------------------|--------------------------------|-----------|------------|------------|------------|-----------|
| Time in Minutes         | Surviving Bacteria in 5.0 c.c. |           |            |            |            |           |
| 0                       | 913,000                        | 1,010,000 | 572,000    | 900,000    | 645,000    | 1,100,000 |
| 10                      | 540,000                        | 580,000   | —          | 990,000    | 360,000    | 403,000   |
| 15                      | —                              | —         | —          | —          | 324,000    | 378,000   |
| 20                      | 308,000                        | 263,000   | —          | 365,000    | 227,000    | 326,000   |
| 25                      | 187,000                        | 145,000   | —          | 155,000    | 198,000    | 189,000   |
| 30                      | 40,000                         | 90,500    | 33,600     | 90,500     | 148,000    | 81,500    |
| 35                      | 6,100                          | 17,700    | 12,400     | 20,800     | 49,000     | 10,900    |
| 40                      | 2,700                          | 900       | 2,700      | 1,900      | 11,000     | 1,700     |
| 45                      | 480                            | < 100     | —          | 350        | 3,100      | 200       |
| 50                      | < x100                         | 0         | —          | 0          | 500        | 0         |
| 55                      | †0                             | 0         | —          | 0          | < 100      | 0         |

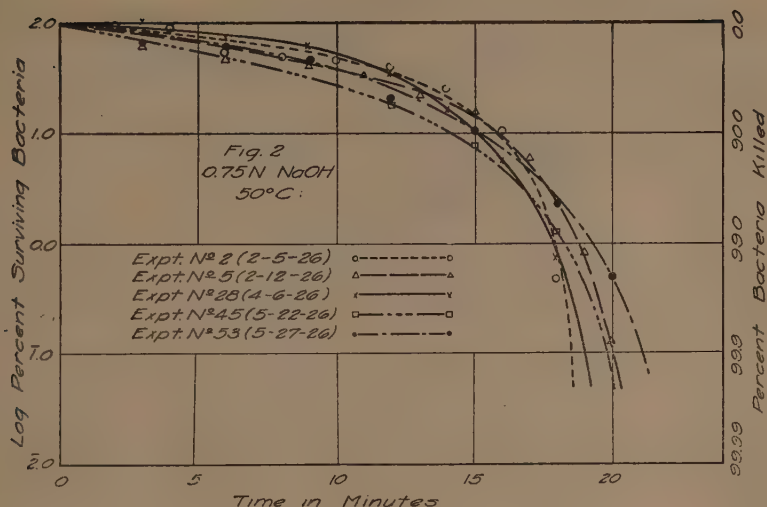
| Expt. No.<br>Date, 1926 | 82<br>8/9 | 86<br>8/12 | 91<br>8/13 | 136<br>9/3 | Av. %<br>Survivors | Log Av. %<br>Survivors |
|-------------------------|-----------|------------|------------|------------|--------------------|------------------------|
| 0                       | 943,000   | 696,000    | 971,000    | 985,000    | 100.0              | 2.000                  |
| 10                      | 438,000   | 327,000    | 518,000    | 357,000    | 55.8               | 1.748                  |
| 15                      | 343,000   | 255,000    | 399,000    | 263,000    | 37.8               | 1.578                  |
| 20                      | 315,000   | 201,000    | 255,000    | 207,000    | 30.5               | 1.486                  |
| 25                      | 161,000   | 148,000    | 144,000    | 151,000    | 18.7               | 1.274                  |
| 30                      | 90,000    | 41,300     | 22,300     | 70,000     | 8.44               | 0.927                  |
| 35                      | 16,300    | 4,700      | 1,800      | —          | 2.09               | 0.322                  |
| 40                      | 3,200     | < 100      | < 100      | 2,580      | 0.36               | 1.557                  |
| 45                      | < 100     | 0          | 0          | 200        | —                  | —                      |
| 50                      | 0         | 0          | 0          | 0          | —                  | —                      |
| 55                      | 0         | 0          | 0          | 0          | —                  | —                      |

x Less than 4 colonies on two plates.

† A recorded count of (0) indicates merely that no colonies developed on plates and not necessarily complete disinfection.

b. *Experiments with 0.75 Normal Sodium Hydroxide at 50°C.*—The results obtained in five experiments using 0.75N sodium hydroxide at 50°C. are given in Table II and shown graphically in Fig. 2. In experiments Nos. 2 and 5 of this series the technique was slightly different from that described above in that the alkali (5 cc. portions) was kept in test tubes to each of which was added the desired quantity of bacterial suspension and at the times specified the contents of the tubes were poured in to 45 cc. of a neutralizing acid solution. This technique which was employed in the first nine experiments was discarded as unsatisfactory in favor of that described at the beginning of this paper, but the few experiments performed are included for completeness. The types of disinfection curves obtained with the two techniques were the same but difficulties were encountered in the test tube series in the process of inoculation.

In general the results are similar to those obtained with the 0.5N alkali. The killing time varied from 18.5 minutes to 21.5 minutes, with an average (see Fig. 10) of 19.8 minutes. The course of disinfection in the individual experiments was similar, the rates of death per cell progressively increasing with time. The curve representing the average course of disinfection shows



that about 41% of the exposed bacteria were killed in the first six minutes; of those remaining 55% died in the second six minute interval, and approximately 94% of the survivors succumbed in the third six minute period.

TABLE II

SHOWING SURVIVING BACTERIA IN 0.75N SODIUM HYDROXIDE AT 50°C.

| Expt. No.<br>Date, 1926 | 2<br>2/5                       | 5<br>2/12 | 28<br>4/6 | 45<br>5/22 | 53<br>5/27 | Av. %<br>Survivors | Log Av. %<br>Survivors |
|-------------------------|--------------------------------|-----------|-----------|------------|------------|--------------------|------------------------|
| Time in Min.            | Surviving Bacteria in 5.0 c.c. |           |           |            |            |                    |                        |
| 0                       | 677,000                        | 1,750,000 | 913,000   | 572,000    | 900,000    | 100.0              | 2.000                  |
| 2                       | 632,000                        |           |           |            |            |                    |                        |
| 3                       |                                | 1,032,000 | 950,000   |            | 563,000    | 75.7               | 1.800                  |
| 4                       | 625,000                        |           |           |            |            |                    |                        |
| 6                       | 355,000                        | 785,000   | 652,000   |            | 530,000    | 54.5               | 1.738                  |
| 8                       | 315,000                        |           |           |            |            |                    |                        |
| 9                       |                                | 687,000   | 580,000   |            | 380,000    | 45.8               | 1.662                  |
| 10                      | 304,000                        |           |           |            |            |                    |                        |
| 11                      |                                | 588,000   |           |            |            |                    |                        |
| 12                      | 273,000                        |           | 313,000   | 104,000    | 178,000    | 28.2               | 1.435                  |
| 13                      |                                | 378,000   |           |            |            |                    |                        |
| 14                      | 168,000                        |           | 145,000   |            |            |                    |                        |
| 15                      |                                | 276,000   |           | 44,800     | 92,000     | 12.7               | 1.104                  |
| 16                      | 72,000                         |           | 50,300    |            |            |                    |                        |
| 17                      |                                | 178,000   |           |            |            |                    |                        |
| 18                      | 3,200                          |           | 6,670     | 7,400      | 20,000     | 1.38               | 0.140                  |
| 19                      |                                | 14,400    |           |            |            |                    |                        |
| 20                      |                                |           | 125       |            |            |                    |                        |
| 21                      |                                |           |           | < 100      | 4,200      |                    |                        |
| 24                      |                                |           |           |            | < 100      |                    |                        |

c. *Experiments with 1.0N Sodium Hydroxide at 50°C.*—The results obtained in four experiments employing 1.0N sodium hydroxide at 50°C.

are given in Table III and shown graphically in Fig. 3. Here it is again observed that the proportion of exposed bacteria killed increased progressively with time. About 37.% were killed during the first four minutes, 54% of the survivors died during the next four minutes, and of those that

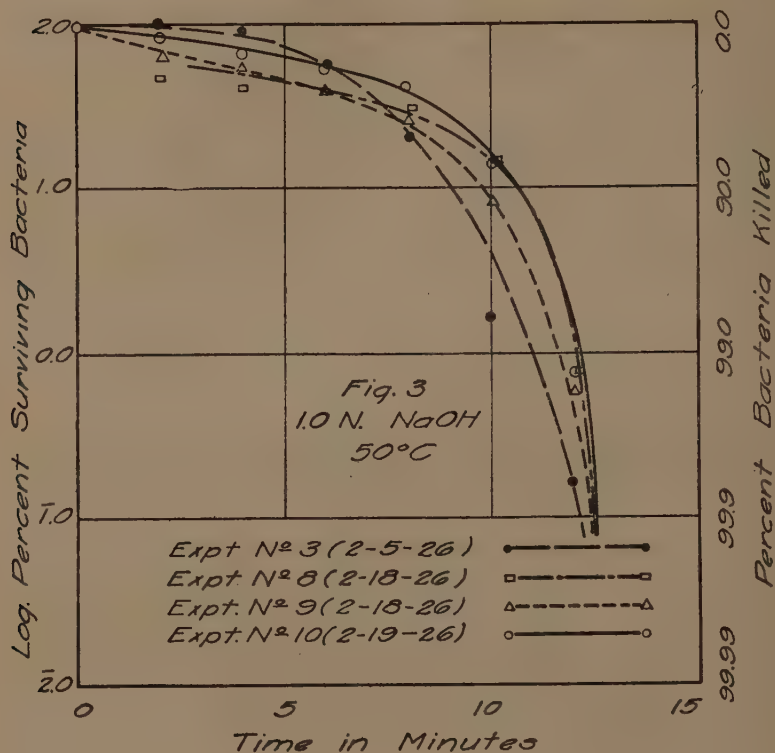


TABLE III

SHOWING SURVIVING BACTERIA IN 1.0N SODIUM HYDROXIDE AT 50°C.

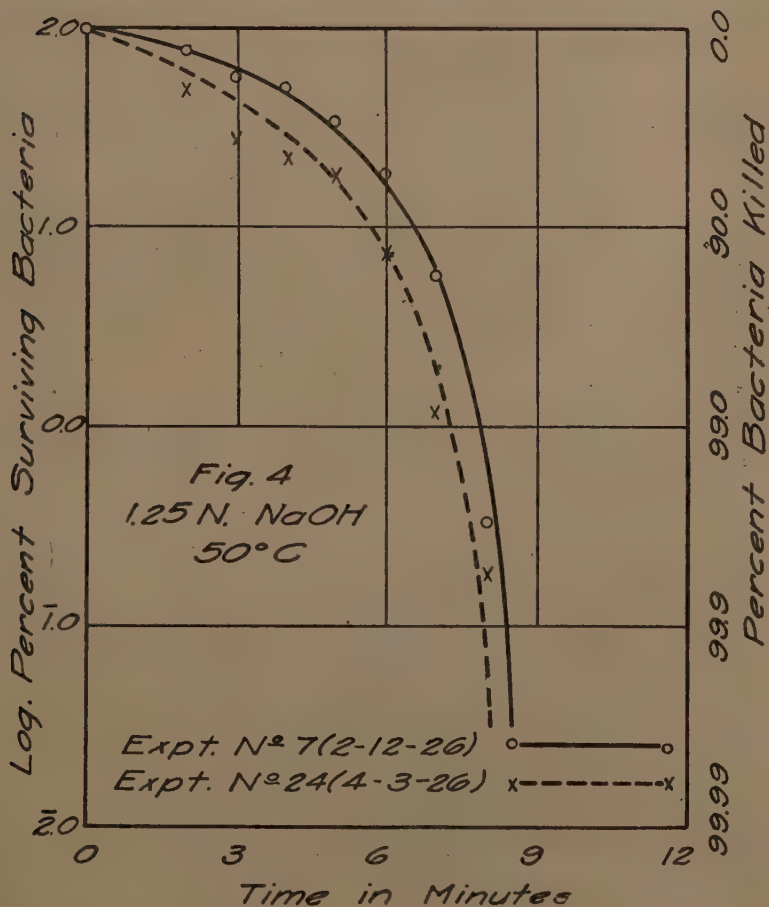
| Expt. No.<br>Date, 1926 | 3<br>2/5                       | 8<br>2/18 | 9<br>2/18 | 10<br>2/19 | Av. %<br>Survivors | Log Av. %<br>Survivors |
|-------------------------|--------------------------------|-----------|-----------|------------|--------------------|------------------------|
| Time in Minutes         | Surviving Bacteria in 5.0 c.c. |           |           |            |                    |                        |
| 0                       | 677,000                        | 1,250,000 | 1,080,000 | 685,000    | 100.0              | 2.000                  |
| 2                       | 680,000                        | 565,000   | 665,000   | 553,000    | 72.1               | 1.859                  |
| 4                       | 585,000                        | 495,000   | 545,000   | 512,000    | 62.8               | 1.798                  |
| 6                       | 375,000                        | 476,000   | 565,000   | 376,000    | 50.2               | 1.702                  |
| 8                       | 144,000                        | 357,000   | 276,000   | 271,000    | 28.8               | 1.460                  |
| 10                      | 11,100                         | 177,000   | 88,500    | 93,500     | 9.1                | 0.960                  |
| 12                      | 1,200                          | 9,700     | 6,200     | 4,950      | 0.56               | 1.749                  |
| 14                      | —                              | 2,000     | —         | < 100      | —                  | —                      |



TABLE IV

SHOWING SURVIVING BACTERIA IN 1.25N SODIUM HYDROXIDE AT 50°C.

| Expt. No.<br>Date, 1926 | 7<br>2/12                      | 24<br>4/3 | Average %<br>Survivors | Log Average<br>% Survivors |
|-------------------------|--------------------------------|-----------|------------------------|----------------------------|
| Time in Minutes         | Surviving Bacteria in 5.0 c.c. |           |                        |                            |
| 0                       | 1,750,000                      | 1,005,000 | 100.0                  | 2.000                      |
| 2                       | 1,330,000                      | 490,000   | 62.4                   | 1.796                      |
| 3                       | 972,000                        | 270,000   | 41.2                   | 1.615                      |
| 4                       | 865,000                        | 220,000   | 35.7                   | 1.554                      |
| 5                       | 580,000                        | 185,000   | 25.8                   | 1.412                      |
| 6                       | 325,000                        | 76,100    | 13.1                   | 1.118                      |
| 7                       | 100,000                        | 12,200    | 3.45                   | 0.539                      |
| 8                       | 6,000                          | 1,800     | 0.26                   | 1.416                      |
| 9                       | < 100                          |           |                        |                            |



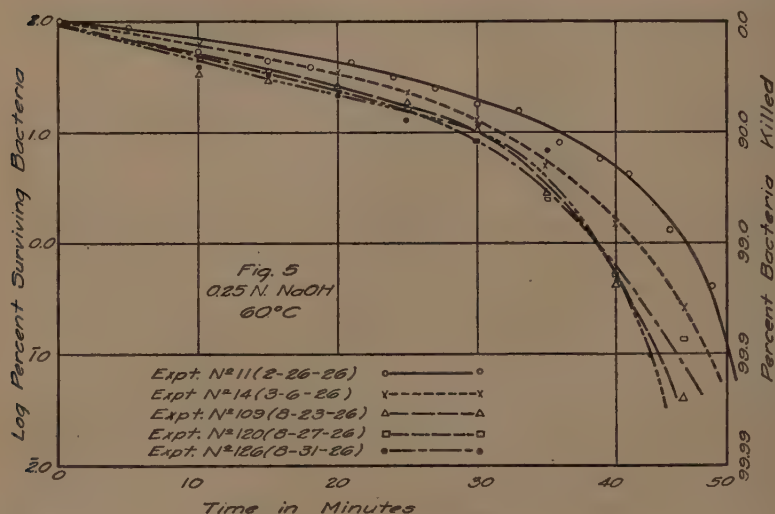
remained after eight minutes over 98% succumbed in the next four minute period of exposure.

The killing time for 99.9% of the bacteria varied from 12.1 to 12.6 minutes with an average of 12.4 minutes.

d. *Experiments with 1.25N Sodium Hydroxide at 50°C.*—Two experiments were run with 1.25N sodium hydroxide at 50°C. The results are given in Table IV and shown graphically in Fig. 4. The two curves are almost parallel and again show an increasing death rate as the period of exposure progresses. The time for effecting a reduction of 99.9% was 8 to 8.4 minutes or an average of 8.2 minutes.

## 2. Effect of Concentration of Sodium Hydroxide on Killing Time at 60°C

Concentrations of 0.25N, 0.5N and 0.75N sodium hydroxide were employed to ascertain the killing time at 60°C. In Table V and Fig. 5 are



given the results for five experiments using 0.25N alkali. The killing time (99.9%) varied from 42.6 minutes to 50.2, the composite curve for all the experiments (Fig. 11) showing an average of 46.8 minutes.

The results obtained in 5 runs using 0.5N sodium hydroxide at 60°C. are given in Table VI and shown graphically in Fig. 6. The killing time varied from 10.4 minutes to 12.3 minutes with an average (Fig. 11) of 11.7 minutes.

Three runs were made with 0.75N caustic soda at 60°C. The results are shown in Table VII and Fig. 7. The disinfection times (99.9%) were 5.0, 5.15 and 6.15 minutes.

In all of the experiments at 60°C. increasing rates of death per cell as the period of exposure progressed were clearly evident. In this respect the results were similar to those observed at 50°C.

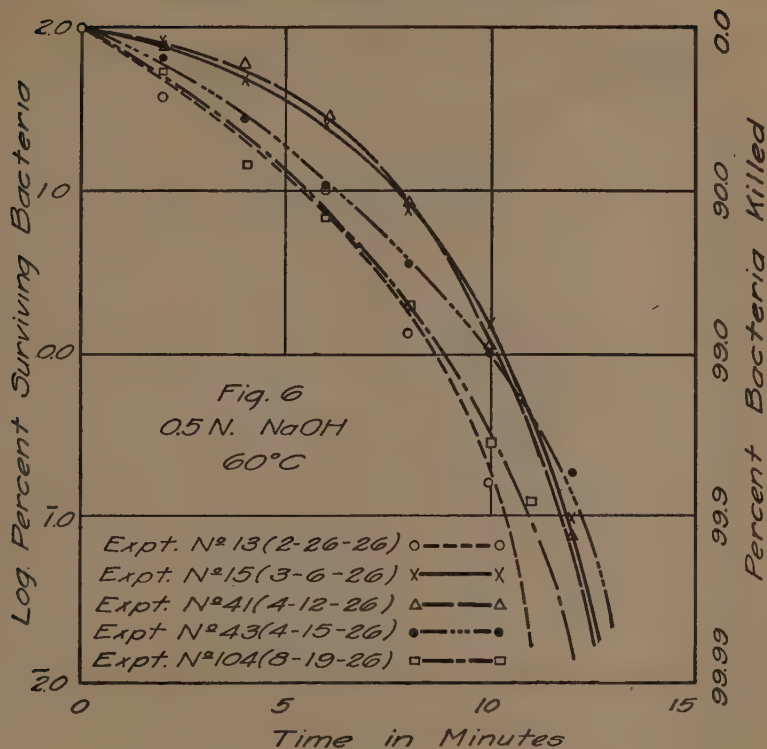


TABLE V

SHOWING SURVIVING BACTERIA IN 0.25N SODIUM HYDROXIDE AT 60°C.

| Expt. No.<br>Date, 1926 | 11<br>2/26                     | 14<br>3/6 | 109<br>8/23 | 120<br>8/27 | 126<br>8/31 | Av. %<br>Survivors | Log Av. %<br>Survivors |
|-------------------------|--------------------------------|-----------|-------------|-------------|-------------|--------------------|------------------------|
| Time in Min.            | Surviving Bacteria in 5.0 c.c. |           |             |             |             |                    |                        |
| 0                       | 959,000                        | 1,036,000 | 1,030,000   | 1,100,000   | 1,020,000   | 100.0              | 2.000                  |
| 5                       | 818,000                        |           |             |             |             |                    |                        |
| 10                      | 508,000                        | 627,000   | 340,000     | 469,000     | 403,000     | 45.8               | 1.662                  |
| 15                      | 413,999                        |           | 298,000     | 394,000     | 341,000     | 36.7               | 1.566                  |
| 18                      | 370,000                        |           |             |             |             |                    |                        |
| 20                      |                                | 365,000   | 270,000     | 275,000     | 139,000     | 28.0               | 1.448                  |
| 21                      | 408,000                        |           |             |             |             |                    |                        |
| 24                      | 308,000                        |           |             |             |             |                    |                        |
| 25                      |                                | 240,000   | 187,000     | 173,000     | 130,000     | 20.5               | 1.312                  |
| 27                      | 246,000                        |           |             |             |             |                    |                        |
| 30                      | 172,000                        | 132,000   | 131,000     | 94,500      | 117,000     | 12.7               | 1.04                   |
| 33                      | 148,000                        |           |             |             |             |                    |                        |
| 35                      |                                | 530,000   | 28,000      | 27,700      | 70,500      | 5.46               | 0.738                  |
| 36                      | 77,300                         |           |             |             |             |                    |                        |
| 39                      | 55,300                         |           |             |             |             |                    |                        |
| 40                      |                                | 15,600    | 4,300       | 5,630       | 4,750       | 1.52               | 0.183                  |
| 41                      | 41,500                         |           |             |             |             |                    |                        |
| 42.5                    |                                |           |             |             | 980         |                    |                        |
| 44                      | 12,700                         |           |             |             |             |                    |                        |
| 45                      |                                | 2,680     | 375         | 1,450       | 125         | 0.30               | 1.478                  |
| 47.5                    | 3,900                          |           |             |             |             |                    |                        |
| 50                      |                                | 350       |             |             |             |                    |                        |

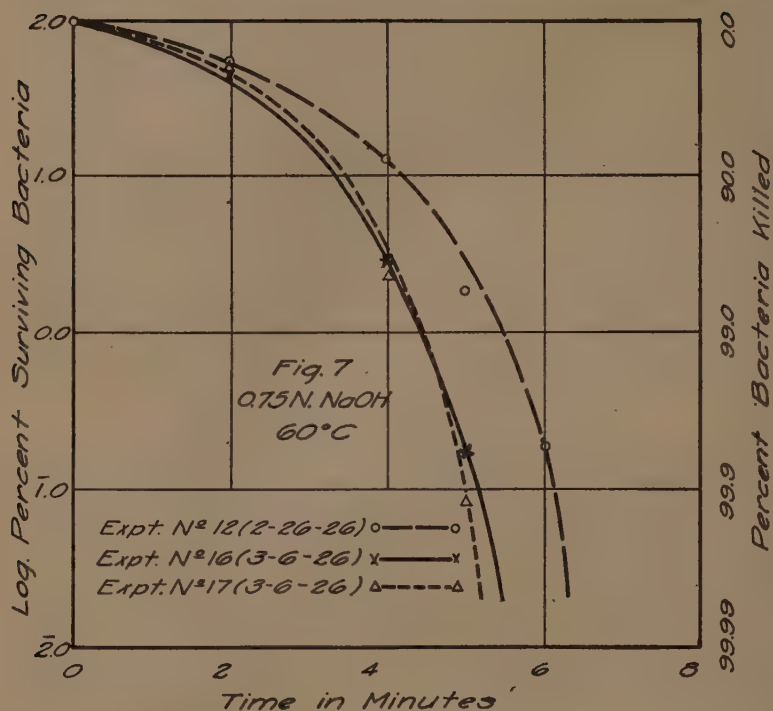


TABLE VI

SHOWING SURVIVING BACTERIA IN 0.5N SODIUM HYDROXIDE AT 60°C.

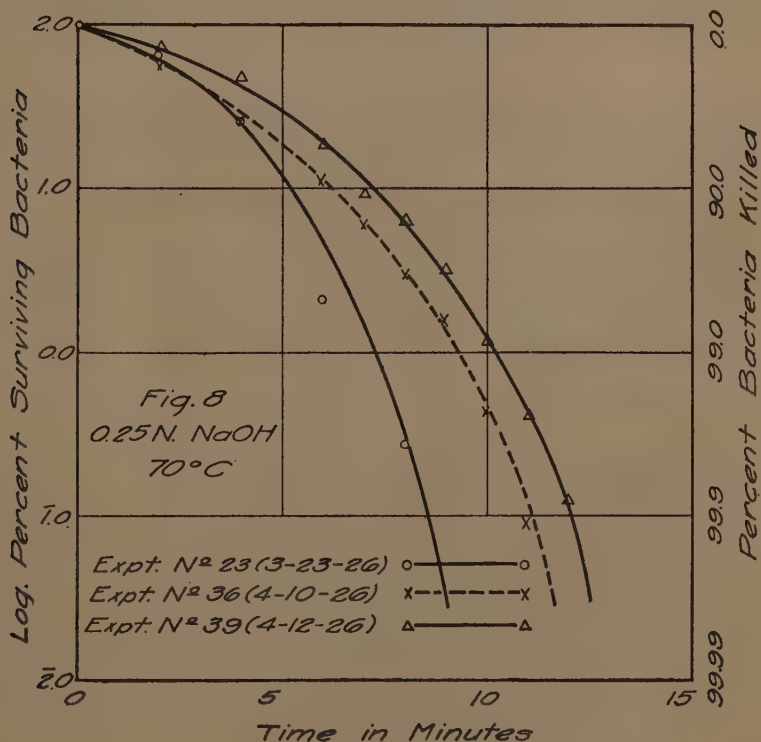
| Expt. No.<br>Date, 1926 | 13<br>2/26                     | 15<br>3/6 | 41<br>4/12 | 43<br>4/15 | 104<br>8/19 | Av. %<br>Survivors | Log Av. %<br>Survivors |
|-------------------------|--------------------------------|-----------|------------|------------|-------------|--------------------|------------------------|
| Time in Min.            | Surviving Bacteria in 5.0 c.c. |           |            |            |             |                    |                        |
| 0                       | 973,000                        | 1,036,000 | 1,050,000  | 881,000    | 653,000     | 100.0              | 2.000                  |
| 2                       | 365,000                        | 845,000   | 813,000    | 558,000    | 343,000     | 62.5               | 1.796                  |
| 4                       | —                              | 495,000   | 675,000    | 246,000    | 129,000     | 35.8               | 1.555                  |
| 6                       | 106,000                        | 262,000   | 288,000    | 92,500     | 48,000      | 16.3               | 1.213                  |
| 8                       | 12,900                         | 82,500    | 82,500     | 32,300     | 12,900      | 4.56               | 0.660                  |
| 10                      | 1,650                          | 15,900    | 17,800     | 9,400      | 2,050       | 0.84               | 1.925                  |
| 11                      | —                              | —         | —          | —          | 825         | —                  | —                      |
| 12                      | 200                            | 1,050     | 800        | 1,700      | 125         | 0.08               | 2.904                  |
| 14                      | 0                              | < 100     | 0          | 150        | 0           | —                  | —                      |

TABLE VII  
SHOWING SURVIVING BACTERIA IN 0.75N SODIUM HYDROXIDE AT 60°C.

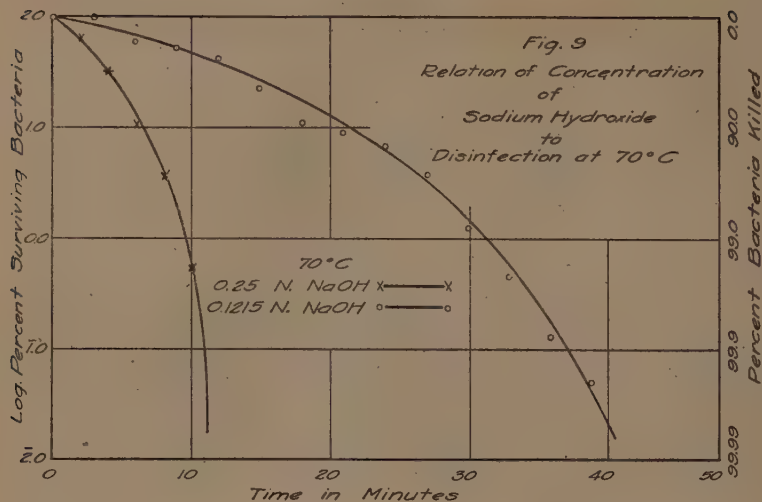
| Expt. No.<br>Date, 1926 | 12<br>2/26                     | 16<br>3/6 | 17<br>3/6 | Average %<br>Survivors | Log Av. %<br>Survivors |
|-------------------------|--------------------------------|-----------|-----------|------------------------|------------------------|
| Time in Min.            | Surviving Bacteria in 5.0 c.c. |           |           |                        |                        |
| 0                       | 973,000                        | 1,036,000 | 1,036,000 | 100.0                  | 2.000                  |
| 2                       | 528,000                        | 450,000   | 525,000   | 49.2                   | 1.692                  |
| 4                       | 122,000                        | 28,600    | 24,100    | 5.86                   | 0.768                  |
| 5                       | 17,600                         | 1,800     | 850       | 0.69                   | 1.840                  |
| 6                       | 1,950                          | < 100     | < 100     | 0.07                   | 2.846                  |
| 7                       | < 100                          |           |           |                        |                        |

### 3. Effect of Concentration of Sodium Hydroxide on Killing Time at 70°C.

Concentrations of 0.1215N and 0.25N sodium hydroxide were employed at 70°C. The results for three experiments with the higher concentration and one with the lower are shown in Tables VIII and IX and Figs. 8 and 9. The killing times for 99.9% of the exposed bacteria were 10.7 and 37.2 minutes for the 0.25N and 0.1215N alkalis, respectively. The increasing







death rates per cell with increased time of exposure, which were observed in the 50°C. and 60°C. series, were also observed in the 70°C. experiments.

TABLE VIII

SHOWING SURVIVING BACTERIA IN 0.25N SODIUM HYDROXIDE AT 70°C.

| Expt. No.<br>Date, 1926 | 23<br>3/23                     | 36<br>4/10 | 39<br>4/12 | Average %<br>Survivors | Log Av. %<br>Survivors |
|-------------------------|--------------------------------|------------|------------|------------------------|------------------------|
| Time in Min.            | Surviving Bacteria in 5.0 c.c. |            |            |                        |                        |
| 0                       | 750,000                        | 1,590,000  | 1,050,000  | 100.0                  | 2.000                  |
| 2                       | 480,000                        | 883,000    | 733,000    | 63.1                   | 1.801                  |
| 4                       | 187,000                        | 400,000    | 455,000    | 31.1                   | 1.494                  |
| 6                       | 15,200                         | 184,000    | 190,000    | 10.6                   | 1.026                  |
| 7                       | —                              | 93,500     | 98,000     | —                      | —                      |
| 8                       | 2,080                          | 46,300     | 67,000     | 3.2                    | 0.560                  |
| 9                       | —                              | 24,300     | 34,500     | —                      | —                      |
| 10                      | < 100                          | 6,730      | 12,200     | 0.53                   | 1.725                  |
| 11                      | —                              | 1,400      | 4,080      | 0.24                   | 1.382                  |
| 12                      | —                              | 325        | 1,230      | —                      | —                      |
| 13                      | —                              | —          | 175        | —                      | —                      |

TABLE IX

SHOWING SURVIVING BACTERIA IN 0.1215N SODIUM HYDROXIDE AT 70°C.

| Time<br>Minutes | Surviving Bacteria<br>in 5.0 c.c. | % Survivors | Log % Survivors |
|-----------------|-----------------------------------|-------------|-----------------|
| 0               | 1,110,000                         | 100.0       | 2.000           |
| 3               | 1,130,000                         | 101.0       | 2.004           |
| 6               | 663,000                           | 59.9        | 1.777           |
| 9               | 570,000                           | 51.5        | 1.712           |
| 12              | 463,000                           | 41.7        | 1.620           |
| 15              | 248,000                           | 22.3        | 1.340           |
| 18              | 120,000                           | 10.8        | 1.033           |
| 21              | 87,800                            | 8.9         | 0.949           |
| 24              | 76,500                            | 6.9         | 0.839           |
| 27              | 40,500                            | 3.62        | 0.559           |
| 30              | 13,600                            | 1.23        | 0.900           |
| 33              | 5,050                             | 0.45        | 1.653           |
| 36              | 1,480                             | 0.13        | 1.114           |
| 39              | 550                               |             |                 |

## Discussion

The results obtained with various concentrations of sodium hydroxide at 50°, 60° and 70°C. indicate clearly that the velocity coefficients of the rates of death were not constant during the course of an experiment, but that they increased with the time of exposure.

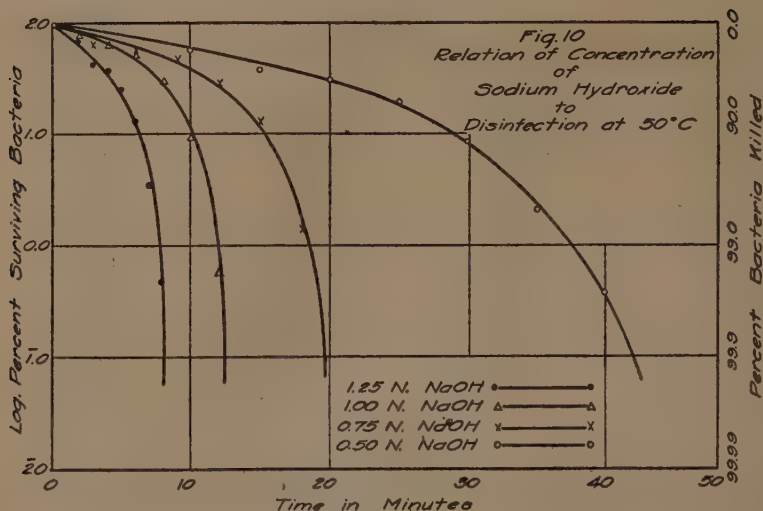
TABLE X

SHOWING RELATION OF TIME OF EXPOSURE TO RATE OF DEATH OF BACTERIA BY SODIUM HYDROXIDE AT 50° TO 70°C.

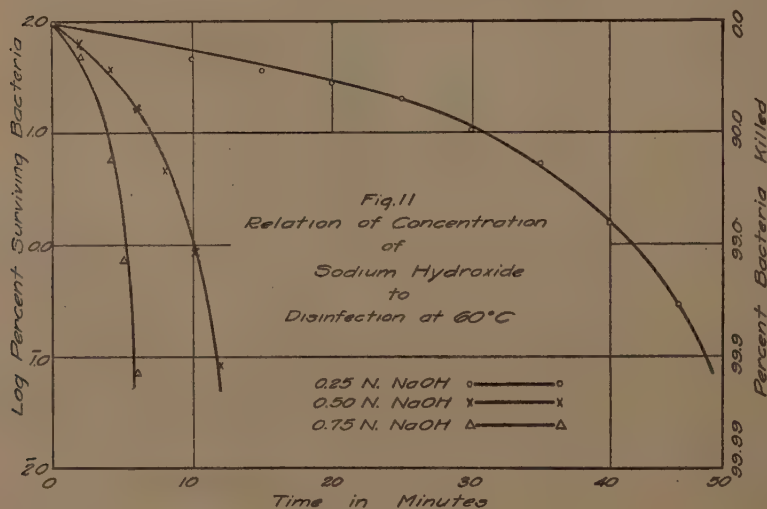
| Elapsed Time<br>in Minutes | 0.1215N NaOH at<br>70°C. |       | 0.25N NaOH at<br>60°C. |       | 0.5N NaOH at<br>50°C. |       |
|----------------------------|--------------------------|-------|------------------------|-------|-----------------------|-------|
|                            | Log %<br>Survivors       | K     | Log %<br>Survivors     | K     | Log %<br>Survivors    | K     |
| 0                          | 2.000                    | —     | 2.000                  | —     | 2.000                 | —     |
| 10                         | 1.662                    | .0338 | 1.738                  | .0262 | 1.770                 | .0230 |
| 20                         | 1.088                    | .0574 | 1.475                  | .0263 | 1.475                 | .0295 |
| 30                         | 0.150                    | .0938 | 1.050                  | .0425 | 0.900                 | .0575 |
| 35                         | 1.413                    | .1474 | 0.688                  | .0724 | 0.375                 | .1050 |
| 40                         | —                        | —     | 0.188                  | .1000 | 1.538                 | .1674 |

This is very well shown in Table X where the velocity coefficient of the death rate, "K," is calculated from the formula for logarithmic death rates ( $K = \frac{1}{t} \log \frac{B_1}{B_2}$ )\* employing the lowest concentrations of alkali at the three temperatures studied. The values for "K" progressively increased until during the last 5 minutes of exposure they were from 4 to 7 times as great as the observed values during the first ten minutes. The hope of employing velocity constants for computing the effects of concentration and temperature on the germicidal efficiency of sodium hydroxide therefore had to be abandoned.

\*  $B_1$  &  $B_2$  are the numbers of surviving bacteria at the beginning and end respectively, of time "t."



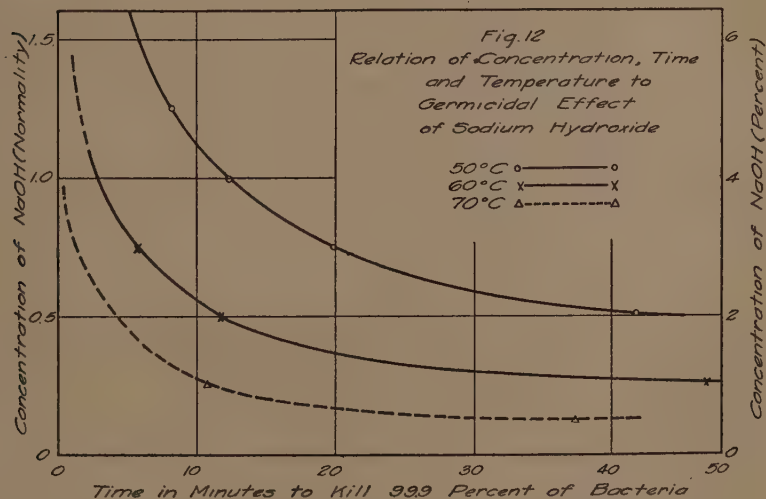
In Fig. 10 are plotted the logarithms of the average per cent surviving bacteria for all experiments at 50°C. against time. These included ten experiments with 0.5N, five runs with 0.75N, four series using 1.0N and two observations employing 1.25N sodium hydroxide. The points fall upon smooth curves with increasing slopes. The abscissae of the points of intersection of these curves with the ordinate 1.000 represent the killing time



of 99.9% of the exposed bacteria. For the series in question the killing times were as follows: 0.5N, 41.7 minutes; 0.75N, 19.8 minutes; 1.0N, 12.4 minutes; and 1.25N, 8.2 minutes.

In Fig. 12 these killing times are plotted against the corresponding concentrations of sodium hydroxide. The points describe a smooth curve from which may be ascertained the time required for effecting a reduction of 99.9% at 50°C. with any concentrations of sodium hydroxide between two and six per cent. By extrapolation the curve may be extended to include a wider range of caustic alkali.

The results of the experiments at 60°C. were treated in a manner analogous to that described for the 50°C. series. As shown in Figs. 11 and



12 the 60°C. observations included five runs with 0.25N, five experiments with 0.5N and three series using 0.75N sodium hydroxide. The 99.9% killing times were 46.8, 11.7 and 5.7 minutes, respectively.

The observations at 70°C. consisted of only one experiment with 0.1215N and three experiments with 0.25N sodium hydroxide, and these showed killing times of 37.4 and 10.7 minutes, respectively. (See Fig. 9). The number of observations is insufficient to permit construction of a dependable curve showing the relation of killing time to concentration of alkali at 70°C. On the basis of the two observed points and the 60° and 50° results a tentative curve for 70°C. is included in Fig. 12.

The effect of change in concentration of alkali or change in temperature on the killing time may be readily ascertained from the curves in Fig. 12.

### SUMMARY

1. A technique is described for measuring the relative germicidal efficiencies of strong alkalis.

2. Sodium hydroxide in concentrations of 0.1215N and 0.25N was studied at 70°C., 0.25N to 0.75N at 60°C., and 0.5N to 1.25N at 50°C., and were found to give concordant results on different days.
3. The velocity coefficients of the rates of death of the exposed bacteria were not constant during the course of an experiment, but on the contrary, progressively increased with the time of exposure.
4. The death or killing time of 99.9% of the exposed bacteria is considered a suitable, determinable, and desirable criterion of the relative germicidal efficiency of the alkali solutions studied.
5. Curves are presented showing the relation of concentration of sodium hydroxide and temperature of exposure to killing time.



# THE NITROGEN METABOLISM OF NITROGEN-FIXING BACTERIA<sup>1</sup>

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The mechanism of nitrogen fixation by bacteria is a problem yet to be solved. Synthetic solutions containing a suitable carbohydrate but no combined nitrogen soon accumulate measurable quantities of combined nitrogen after being inoculated with certain bacteria. It is commonly assumed that the carbohydrates supply a source of energy to the organism, enabling it to utilize the nitrogen of the air in some way not yet understood.

Chemical analyses of cultures of nitrogen-fixing bacteria grown in a nitrogen-free, synthetic medium should indicate the compounds of nitrogen produced in their metabolism. Such analyses made on cultures at different periods of growth may indicate the trend of synthesis by demonstrating successive steps, or there may be no accumulation of intermediate products at any time and thus present a riddle comparable to that of photosynthesis.

## Historical

Stoklasa, Ernest, Stranak, and Vitek (21) showed that traces of certain nitrogen compounds stimulate the growth of *Azotobacter* cultures, but larger quantities reduce the amount of fixation and may themselves be changed. They demonstrated that these organisms do not lose the power to utilize atmospheric nitrogen by cultivation on nitrogen-containing media. That legume bacteria do not lose their power to form nodules through cultivation on a nitrogen-containing medium has been shown by Burrill and Hansen (3). Golding (6) found that the fixation of nitrogen ceases unless the resulting compound is removed as in the plant. Hills (9) demonstrated that *Azotobacter* behaved differently toward different nitrates. Potassium nitrate and sodium nitrate increased the amount of nitrogen fixed in agar film cultures, whereas, calcium nitrate in the same concentration decreased the amount.

If the legume bacteria in the root nodule fix atmospheric nitrogen merely to supply their own needs, it would seem logical to conclude that (1) the first products formed are simple, soluble compounds for which the leguminous plant and the bacteria compete, or (2) the plant secretes an enzyme which decomposes the bacterial cell, thus making the nitrogen available, or (3) autolysis of the bacterial cell supplies the host plant with soluble nitrogenous compounds.

That the first explanation is the most probable is suggested by the following references. Whiting and Schoonover (24) have shown that 26 days after planting inoculated cowpea seedlings in purified nitrogen-free sand, the nitrogen fixed was three times that contained in the seeds planted.

<sup>1</sup> A dissertation submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy, Iowa State College.

They maintain that the fixation of nitrogen in cowpea seedlings is closely associated with the supply of available carbohydrates within the plant. Since the bacteria on entering the root hairs find favorable conditions for growth, it does not seem possible that within so short a time digestion of the bacterial cell could take place through the action of enzymes secreted by the leguminous plant or the bacteria themselves, so as to supply soluble cleavage products of the protoplasmic protein. Lipman (16) explains, however, that "the organisms that find their way into the legume roots multiply there rapidly and increase to enormous numbers. The small rod-shaped forms are rather abundant in the young tubercles, but as the latter grow older, the larger irregular forms—the bacteroids—become more and more numerous. Later still, the bacteroids are dissolved and absorbed by the host plant." Golding (6) concluded that plant enzymes do not play an important part in the assimilation of nitrogen and that one of the functions of the host plant is the removal of soluble products of growth.

Neither is it probable that autolysis could account for the formation of soluble nitrogen because cultures of *Rhizobium leguminosarum* and *Azotobacter* retain their vitality for years when grown on a suitable medium. It is generally assumed that bacteria gain entrance to the root hair by secreting an enzyme that dissolves the cell wall. The nature of this enzyme, however, has never been determined. Pierce (19) states, "the tubercle bacteria enter and infect a root hair by softening or dissolving a small portion of the wall and moving or growing through this. There is no evidence that they usually enter through broken root hairs, etc.". Under these conditions, the bacteria within the nodules would fix nitrogen in a soluble form. The affinity of the host plant for this nitrogen would be so great that there would be an actual competition between the bacteria and the plant. The bacteria would then retain only enough nitrogen to build up their cells and there would be no accumulation of nitrogen within the nodule other than that stored up in the bodies of the bacteria. If this condition actually obtains, sterile plants growing in a synthetic medium containing an abundance of suitable carbohydrate for bacterial growth, would not readily form nodules when the medium was inoculated with nodule-forming bacteria, because the bacteria would find more favorable conditions for growth in the medium than within the plant cells, and the plants would show all the benefits of inoculation. Joshi (13) obtained increased growth of plants where the bacteria were confined within porous cylinders; non-leguminous plants were greatly benefited by inoculation with legume bacteria and *Azotobacter*.

*Theories Concerning the Mechanism of Nitrogen Fixation.* Theories of the mechanism and chemical reactions in the fixation of atmospheric nitrogen by bacteria are at variance. That the various hypotheses are plausible is evidenced by the fact that nitrogen fixation is actually effected on a commercial scale according to these theories. The conditions for nitrogen fixation require that the inert nitrogen of the atmosphere become reactive. This molecular change requires energy. Linhart (15) estimates that only one percent of the total available energy is used by *Azotobacter* for the fixation of nitrogen in a mannitol medium.

Gautier and Drouin (4) believed that the organisms oxidized free nitrogen to nitrous and nitric acid, which were subsequently reduced to an ammonium compound and further changed to other nitrogenous compounds. Support of this theory is offered in the fact that Stoklasa (20)

found nitrates in the nodules of leguminous plants. On the contrary Whit-  
ing (25) failed to find nitrite, nitrate, or ammonia in the nodules or in  
plants grown in a nitrogen-free medium.

Heinze (8) reports having detected hydro-carbons of the acetylene series  
in crude nitrogen-fixing cultures. He gives an equation showing com-  
bination of acetylene and free nitrogen to form hydrocyanic acid, which  
would undergo immediate transformation into ammonium formate. Ger-  
lach and Vogel (5) accept this theory—that a direct combination of free  
nitrogen with carbon compounds takes place inside the cell. However,  
Strowd (23) could not detect cyanides in the extract from 100-gram quan-  
tities of soy bean nodules even by a method sensitive to 0.01 mgs. hydro-  
cyanic acid.

Lipman (17) suggests the feasibility of the course of synthesis as pass-  
ing through the oxyacids, amino acids, peptides, polypeptides, and so forth.

Winogradsky (26) believed that ammonia forms by direct union of  
free nitrogen and nascent hydrogen. Stoklasa (22) agrees in this.

Loew and Aso (18) suggest that ammonium nitrite is formed accord-  
ing to the equation  $N_2 + 2H_2O = NO_2NH_4$ .

These theories in general assume that the nitrogen is fixed in a soluble  
form. Gerlach and Vogel (5) question the theory advanced by Beijerinck  
and Van Delden that a soluble nitrogen compound was first formed by  
*Azotobacter*, by showing that no soluble nitrogen was present in cultures  
following fixation.

Lipman (17) found that in the course of nitrogen fixation by *Azoto-  
bacter vinelandii* a soluble nitrogenous substance is formed which is not  
an essential part of the bacterial cell and which diffuses outward into the  
surrounding medium. "This compound," he observes, "may be an albu-  
mose, but not necessarily in itself the first product of synthesis."

*Nitrogen Determinations on Azotobacter Cultures.* Chemical analyses  
have been made on *Azotobacter* cultures by several investigators. These  
analyses were made on cultures grown under a wide range of conditions,  
but they all point to the fact that only part of the nitrogen present is in  
the form of protein and generally the older cultures are higher in protein.  
Lipman (17) reports the nitrogenous composition of dried *Azotobacter*  
membrane as 10.45 percent total nitrogen, 6.39 percent non-basic nitrogen,  
2.76 percent basic nitrogen, and 0.98 percent ammonia nitrogen.

Hoffman and Hammer (10) grew *Azotobacter chroococcum* on Ashby's  
solution to which had been added 1.5 percent agar. The cells were scraped  
off the surface with a clean glass slide, dried and pulverized before the  
nitrogen determinations were made. *Azotobacter* cells, incubated for seven  
days before drying, contained 1.86 percent nitrogen whereas dry cells from  
21-day incubation showed 2.84 percent nitrogen. Calculated as protein  
these would be equivalent to 11.62 percent and 17.75 percent protein respec-  
tively. No attempt was made to partition the various kinds of nitrogen.  
These figures are in agreement to those given by Gerlach and Vogel (5),  
who report a protein content of 8.0 percent, and by Stoklasa who reports a  
protein content of 11.3 percent. They show that wide variations in the  
nitrogen content of dry bacterial cells are not uncommon.

Hunter (11) analyzed *Azotobacter* cells scraped from dextrose-Ashby's  
agar. The cultures had been incubated for two to four days at 30°C. The  
cells were air dried. Analysis showed an average of 3.73 percent, which  
calculated as protein, would be 23.31 percent. An analysis of a composite

sample gave 3.55 percent total nitrogen; 1.89 percent albuminoid nitrogen. Results indicate that only 53.1 percent of the total nitrogen is protein, giving a protein content of 11.81 percent instead of 23.31 percent as calculated from the total nitrogen. Organisms grown in liquid cultures aerated from two to four days at 30°C. in dextrose-Ashby medium were removed from the liquid in a super-centrifuge and dried. The composition was: total nitrogen 5.15 percent; albuminoid nitrogen 4.89 percent. Albuminoid nitrogen equaled 94.9 percent of total nitrogen. This indicates a protein content of 30.56 percent as compared with 11.81 percent for the growth obtained from Ashby's agar.

It would have thrown much light on our present problem if an analysis, in which *Azotobacter* cells grown for several weeks under the same conditions, had been reported. This work, however, brings out the point previously emphasized by Lipman, that young cultures of *Azotobacter* contain relatively large percentages of non-protein nitrogen long before any appreciable autolysis has taken place. In the light of these analyses it seems as though this non-protein nitrogen is probably the source from which the bacteria build up the protein of their cells.

*Studies on the Physiology of the Legume Bacteria.* The physiology of nitrogen fixation was investigated by Greig-Smith (7), who reported a study of the growth habits of the legume bacteria and of the gum produced by them in various combinations of nutrients. He deduced that the slime produced is closely related to the nucleoprotein molecule, that in nature the slime is partly used *in situ* by the plant and that the slime may be transferred to other parts of the plant where it is used. He concluded that the slime produced is in ratio to the nitrogen fixed.

Buchanan (2) made a study of the gum produced by "*B. radicicola*" (*Rhizobium leguminosarum*.) He inferred that all nodule bacteria studied formed gum which when produced in saccharose was closely related to the dextrans. He said that gum is not a source of nitrogen to the plant, but a capsule surrounding the cell. Two percent saccharose was the optimum carbohydrate concentration to produce the most gum.

More recently Joshi (13) made a study on the root nodule organism of the leguminous plants. He obtained increased growth of plants from inoculation with root nodule organisms, although nodule formation did not always occur. In cases where no nodules occurred he suggested (1) that the nitrogen might be fixed non-symbiotically in the sand and (2) that the organisms may have been present in the root without the formation of nodules. A search failed to show the presence of the organisms in root tissues. Further tests in which the organisms were confined within porous cylinders showed that the plants inoculated with their own strain of bacteria did better than with cross inoculation. Those inoculated with *Azotobacter* came third, being better than those in which the organisms were confined in a cylinder. Non-leguminous plants behaved in general as leguminous plants. It is surprising that the roots of oats should make greater development in sand inoculated with pea nodule bacteria than with *Azotobacter*, when it is usually recognized that *Azotobacter* is much more efficient as a nitrogen-fixer. Analyses of the culture sand showed that assimilation by the plant and nitrogen fixation go on approximately at the same rate. This had been noted by Golding (6), who procured large gains of nitrogen by root nodule organisms, by filtering off the soluble products of growth which prevent assimilation of nitrogen in artificial cultures.



Joshi, studying further the possibility of ammonification and nitrification occurring, found in peptone water after 72 hours about four milligrams of nitrogen as ammonia, but no nitrites or nitrates could be detected in Omelianski's solution. "Since some ammonia is formed from peptone by the nodule organism," he suggests, "it is possible that it may also transform some of the soluble protein produced by its activity."

*Object of These Investigations.* The following studies have been undertaken to throw some light on the mechanism of the fixation of atmospheric nitrogen by bacteria. The literature on this subject is contradictory, the only general agreement being that bacteria do fix appreciable quantities of nitrogen when grown in a suitable culture medium.

In order to proceed with a study of the mechanism of nitrogen fixation the pertinent questions which we must answer are:

1. Will *Azotobacter chroococcum* and *Rhizobium leguminosarum* fix enough nitrogen in artificial media during a short incubation period to permit the use of analytical chemical methods for quantitative and qualitative chemical determinations?

2. What type of medium should be employed?

3. What chemical methods are best adapted to this type of work?

4. What periods of incubation and methods of propagation should be followed to determine the course of nitrogen synthesis?

The proper carrying out of tests should give us information that will warrant a decision as to whether the first product of synthesis is the same for *Rhizobium leguminosarum* as for *Azotobacter chroococcum*. Is the first product of synthesis some soluble diffusible form equally available to the leguminous plant and the bacterial cell, or is it possible that an enzyme is secreted by the leguminous plant or the bacteria themselves which causes digestion of the bacterial cell and thus supplies soluble nitrogen to the plant?

#### Investigational

*Methods:* The chemical methods used were essentially those employed by Kendall (14).

Total nitrogen (Gunning modification of the Kjeldahl method). To duplicate 50 c.c. portions of the bacterial suspension in 500 c.c. Kjeldahl flasks, 5 grams of potassium sulphate and 5 drops of a 10 percent copper sulphate solution and 15 c.c. of nitrogen free sulphuric acid were added before digesting. The nitrogen was liberated by using sodium hydroxide and distilled by aeration into 250 c.c. receiving flasks containing 30 c.c. of N/50 HCl and 125 c.c. of water.

Non-protein nitrogen was determined according to Kendall's modification of the method of Folin. 120 c.c. of bacterial suspension were diluted with 10 c.c. of distilled water and thoroughly mixed. Exactly 10 c.c. of a 10 percent aqueous solution of sodium tungstate were added and thoroughly shaken, then 10 c.c. of 2/3 normal sulphuric acid were added and shaken thoroughly. A drop of congo red was added. It was found necessary to add more sulphuric acid to those cultures having an excess of lime before the desired acid reaction was obtained. The gum in some of the *Rhizobium leguminosarum* cultures still remained in suspension as a viscid mucilaginous mass. Several methods were tried to change its consistency. The one finally adopted was to place the beakers containing the mixture in a water bath and bring the temperature of the bath to boiling. At this point



the gum went into solution and the liquid filtered readily through a medium coarse filter paper. Duplicate 50 c.c. aliquots of the filtrate were placed in Kjeldahl flasks, and digested and otherwise treated as in the test for total nitrogen.

Ammonia nitrogen was determined by placing 20 c.c. of the suspension of organisms in the aeration flasks to which was added a saturated solution of sodium carbonate and sodium oxalate. The liberated ammonia was distilled by aeration into receiving flasks containing N/50 sulphuric acid.

Amino nitrogen (Sorenson). 50 c.c. of culture medium in a beaker were adjusted to the neutral point of phenolphthalein by the cautious use of N/50 NaOH and N/50 HCl. Five c.c. of formaldehyde exactly neutral to phenolphthalein were added. In the presence of  $\text{NH}_2$  groups the solution becomes acid. A second titration to neutrality gives the acidity of the carboxyl groups. Since one free  $\text{COOH}$  occurs for one free  $\text{NH}_2$  group in most amino acids, the equivalent amount of standard ammonia corresponding to the amount of standard alkali in the titration after the addition of the neutral formaldehyde solution is a measure of the amino nitrogen of the solution. This was also checked by the Van Slyke method.

Protein nitrogen is calculated as the difference between the total nitrogen and the non-protein nitrogen.

Nitrites. Trommsdorf's reagent was used to detect the presence of nitrites.

Nitrates. Diphenylamin reagent was used to determine the presence of nitrates.

In order to obtain information concerning the relative amounts of several nitrogenous groups, analyses were made on some cultures that had been grown quickly under optimum conditions and on others that had grown slowly over a long period of time. The results of these determinations are presented in Table I.

In order to accelerate the growth of the bacteria in the young cultures analyzed, they were vigorously aerated during the incubation period. The *Rhizobium* cultures four days old in Moore's medium showed a marked viscosity, while the eight-day cultures were mucilaginous.

The amounts of nitrogen fixed within the eight-day periods are so small for the *Rhizobium* cultures that the experimental error is often greater than the amount of the determination.

Young *Azotobacter* cultures are better adapted to analytical studies because of the larger quantities of nitrogen fixed and because of a smaller amount of capsule material. The old cultures contained larger quantities of total nitrogen. These cultures had been in an incubator for  $2\frac{1}{2}$  years and according to the check had absorbed a certain amount of ammonia from the atmosphere.

In precipitating the proteins, the bacterial cells coagulate into a mass and carry out much of the non-protein material that they have in their cells. For this reason the protein nitrogen determinations must certainly be too high, and for the same reason the non-protein nitrogen would be too small. In order to get an accurate amino nitrogen determination it would be necessary to rupture the cells. In the *Rhizobium* cultures the gum interferes with the filtration. In the old cultures of *Rhizobium* this gum was so thick that the culture could not be poured from the flask. Difficulty is also encountered in making amino nitrogen determinations. The amount of nitrogen in the young cultures of *Rhizobium* is so small as to make the

TABLE 1

## NITROGEN DETERMINATIONS ON CULTURES

The following analyses show the relative ratios of several nitrogenous groups

|  | Nitrogen fixed | Protein nitrogen  |         | Non-protein nitrogen |                           | Amino Nitrogen (Sorenson's method) |                           | Ammonia           |                           | Nitrate Nitrite |
|--|----------------|-------------------|---------|----------------------|---------------------------|------------------------------------|---------------------------|-------------------|---------------------------|-----------------|
|  |                | Mgs. per 100 c.c. | Percent | Mgs. per 100 c.c.    | Percent of total nitrogen | Mgs. per 100 c.c.                  | Percent of total nitrogen | Mgs. per 100 c.c. | Percent of total nitrogen |                 |
|  |                |                   |         |                      |                           |                                    |                           |                   |                           |                 |
| <i>(Rhizobium leguminosarum in Moore's Medium)</i>                                     |                |                   |         |                      |                           |                                    |                           |                   |                           |                 |
| 2 days   | 1.232          | 112               | 9.08    | 1.008                | 81.8                      | —                                  | —                         | .112              | 9.1                       | —               |
| 4 days   | 1.568          | .560              | 35.80   | 1.008                | 64.3                      | —                                  | —                         | .000              | 0.0                       | —               |
| 8 days   | 1.680          | .504              | 33.00   | 1.176                | 70.3                      | —                                  | —                         | .000              | 0.0                       | —               |
| <i>(Rhizobium leguminosarum grown in Dextrose-Ashby's solution)</i>                    |                |                   |         |                      |                           |                                    |                           |                   |                           |                 |
| 1 day  | .210           | 0.000             | 0.00    | .070                 | 0.0                       | .000                               | .00                       | .141              | 0.0                       | —               |
| 2 days   | .336           | .126              | 37.50   | .210                 | 62.5                      | .000                               | .00                       | .141              | 41.7                      | —               |
| 4 days   | .338           | .048              | 14.20   | .290                 | 85.8                      | .000                               | .00                       | .210              | 62.2                      | —               |
| 8 days   | 1.170          | .960              | 82.20   | .210                 | 18.0                      | .224                               | 19.10                     | .210              | 18.0                      | —               |
| <i>(Azotobacter chroococcum in Dextrose-Ashby's solution)</i>                          |                |                   |         |                      |                           |                                    |                           |                   |                           |                 |
| 1 day  | 0.210          | 0.000             | 0.00    | 0.000                | 0.0                       | .000                               | .00                       | .140              | —                         | —               |
| 2 days   | 5.070          | 4.550             | 89.60   | .525                 | 10.4                      | .304                               | 6.00                      | .560              | 11.0                      | —               |
| 4 days   | 10.300         | 9.810             | 96.20   | .490                 | 4.8                       | .216                               | 2.10                      | .560              | 5.4                       | —               |
| 8 days   | 9.410          | 3.740             | 40.00   | 5.670                | 60.2                      | .448                               | 4.70                      | .280              | 3.0                       | —               |
| <i>(Azotobacter and Rhizobium cultures 2½ years old in Dextrose-Ashby's solution)*</i> |                |                   |         |                      |                           |                                    |                           |                   |                           |                 |
| <i>(Van Slyke Method)</i>  |                |                   |         |                      |                           |                                    |                           |                   |                           |                 |
| 1. Rhizobium (no lime)   | 3.360          | 1.110             | 33.00   | 2.250                | 67.0                      | 1.060                              | 32.00                     | .280              | 8.3                       | —               |
| 2. Rhizobium (excess of lime)  | 9.860          | 6.610             | 67.00   | 3.250                | 33.0                      | 2.120                              | 22.00                     | .550              | 5.6                       | —               |
| 3. Azotobacter (no lime)   | 29.600         | 22.110            | 74.70   | 7.490                | 25.3                      | 6.100                              | 21.00                     | 3.080             | 10.4                      | —               |
| 4. Azotobacter (excess of lime)  | 26.880         | 30.040            | 67.10   | 8.740                | 32.9                      | 6.110                              | 23.50                     | 4.330             | 16.1                      | —               |
| 5. Check   | 3.140          | —                 | —       | 2.920                | 93.0                      | 0.000                              | 00.00                     | .42               | 13.4                      | —               |
| 6. Rhizobium (washed off fresh cultures on Moore's agar)                               | 5.820          | 2.330             | 40.00   | 3.490                | 60.0                      | 1.890                              | 32.00                     | .280              | 4.8                       | —               |

\* Considerable evaporation had taken place and the cultures were not made up to the original volume before analyzing.

TABLE II  
TEST FOR NITRATES, NITRITES AND AMMONIA

| Days<br>culture<br>was<br>grown | Rhizobium   |                      | Azotobacter  |                      | Azotobacter aerated                                      |                      |
|---------------------------------|---|----------------------|--|----------------------|--|----------------------|
|                                 | Mgs. of N<br>as $\text{NH}_3$ per<br>100 c.c.<br>culture* | Nitrates<br>Nitrites | Mgs. of N<br>as $\text{NH}_3$ per<br>100 c.c.<br>culture | Nitrates<br>Nitrites | Mgs. of N<br>as $\text{NH}_3$ per<br>100 c.c.<br>culture | Nitrates<br>Nitrites |
| Check                           | .252  | None                 | .224   | None                 | .140   | None                 |
| 1 day                           | .224  | None                 | .168   | None                 | .504   | None                 |
| 2 days                          | .224  | None                 | .168   | None                 | .224   | None                 |
| 4 days                          | .168  | None                 | .168   | None                 | .224   | None                 |

\* The ammonia was determined here by distilling over magnesium oxide.

Van Slyke method impracticable and the capsule material about the *Rhizobium* cells is so impervious that it takes several days for the formaldehyde to penetrate, thus lessening the value of the Sorenson method. In the series of *Rhizobium* cultures in Moore's solution the formaldehyde was allowed to stand in contact with 100 c.c. of culture and in two days' time there was no indication of acidity. Van Slyke's method was used on the old cultures containing more nitrogen. Subsequent studies, however, give more accurate information on the amino nitrogen relations.

A study of the data in Table I makes it apparent that nitrates and nitrites were not present in any of the cultures. Many tests on cultures of *Azotobacter* and *Rhizobium* grown on several kinds of media and under a wide range of conditions have failed to reveal the presence of nitrates or nitrites. Ammonia appears on the first or second days and during the most rapid period of growth the largest amount is present even though the fixed nitrogen is several times greater on the later date. This is further shown in the following results: (Table II)

The accumulation of ammonia in the culture during the period of most rapid growth would suggest that it is being formed more rapidly than it is synthesized into higher nitrogenous compounds. Whether the maximum ammonia content is attained within two days or four days depends on conditions in the culture, such as amount of inoculation, temperature, etc. No such accumulation of ammonia is observed in young cultures that are grown without aeration. In an unaerated solution the organisms grow slower and the ammonia is apparently synthesized as fast as it is formed and any ammonia already present is taken up by the organisms.

The amino nitrogen content of the bacterial cells can not be readily determined. These products being formed within the cell protoplasm, it would be natural to suspect that only a part would diffuse into the surrounding medium, because they would be synthesized at about the same rate that they were formed. Only in young cultures, during the period of most rapid growth would one expect an excess production of amino nitrogen. To check further on this point titrations were made by the Sorenson method on cultures of *Rhizobium* and *Azotobacter*. The samples were then covered with watch glasses and at regular intervals enough N/50 NaOH was added to restore the end point of the phenolphthalein. The following table shows the total number of c.c. of N/50 NaOH used at the various intervals: (Table III)

These cultures had been aerated vigorously during growth. Here it is found that the *Azotobacter* culture had produced a larger quantity of amino nitrogen than the *Rhizobium* culture. As the formaldehyde remained in contact with the cells the reactions progressed, showing that the rate of penetration of formaldehyde into bacterial cells is slow. Further observations showed that for two days an equilibrium had been reached, and very little more acidity developed. It must be admitted that the presence of carbon dioxide is a bad factor, but the comparative value of the results is no less significant. The greater amount of amino nitrogen in the four-day *Azotobacter* culture over the eight-day culture would suggest the possibility that this culture had reached the stage where amino production and synthesis of proteins were more nearly in equilibrium.

Qualitative protein tests were made on all the cultures whose analyses are reported in Table I using several methods that are commonly believed

TABLE III  
THE RATE OF REACTION WITH FORMALDEHYDE

|             | Age of Culture | Amount of culture used | 1st Titration<br>c.c. N/50<br>NaOH used | After 8 hrs.<br>total N/50<br>NaOH used | After 24 hrs.<br>total N/50<br>NaOH used | After 2 days<br>total N/50<br>NaOH used |
|-------------|----------------|------------------------|---|---|--|---|
| Azotobacter | 1 day          | 100c.c.                | 0.8                                     | 1.8                                     | 3.0                                      | 4.2                                     |
|             | 2 days         | 100                    | 1.6                                     | 5.4                                     | 7.4                                      | 8.9                                     |
|             | 4 days         | 100                    | 1.2                                     | 4.0                                     | 7.4                                      | 9.4                                     |
|             | 8 days         | 100                    | 0.8                                     | 2.8                                     | 4.2                                      | 6.0                                     |
| Rhizobium   | 1 day          | 100                    | 0.4                                     | 1.0                                     | 2.0                                      | 3.0                                     |
|             | 2 days         | 100                    | 0.3                                     | 0.6                                     | 1.2                                      | 1.6                                     |
|             | 4 days         | 100                    | 0.3                                     | 1.0                                     | 2.0                                      | 3.2                                     |
|             | 8 days         | 100                    | 0.8                                     | 2.8                                     | 4.0                                      | 5.2                                     |

to indicate the recognized protein linkages. The results of these tests are shown in Table IV.

It would only be logical that the Biuret test should be uniformly positive since this reaction is characteristic of the proteins and some of the more complicated polypeptides. That the Biuret reaction was negative with the first set of cultures is probably because of heavy capsules of gum about the cells.

In all these cultures except the first group, the reagent was allowed to stand in contact with the culture for several hours and the typical violet color came very slowly. Not until an hour after the copper sulphate had been added could it be distinguished beyond doubt in the two-day culture.

The Hopkins-Cole reaction is due to the presence of tryptophane. It is significant that the only positive tests were those made on the 2½ year old cultures of *Azotobacter*.

The bromine test being negative eliminated the possibility of phenol or cresol being present to account for the reaction with Millon's reagent.

Millon's test is positive on all cultures that have grown two days or more. This reaction indicates the presence of an aromatic substance containing a hydroxyl group attached to the benzene ring. This reaction may safely be interpreted to indicate the presence of tyrosine or the hydroxy-phenol group in the protein molecule or the presence of oxyacids. It may be that Millon's is more delicate in the presence of capsule material than the Biuret test and there may be amino acids containing the phenol radical which have not yet been synthesized that have given the test.

The Xanthoproteic reaction is uniformly positive on the *Azotobacter* cultures; occasionally a culture of *Rhizobium* will give a positive reaction. This reaction indicates the presence of the phenyl group. This phenyl group may be either in the protein molecule or in the form of oxyacids. The failure to give consistent results on the *Rhizobium* cultures is probably due to the small quantities of protein or oxyacids present and the large amount of gum.

Morner's reaction is uniformly negative. It is a specific reaction for tyrosine. Either the test is not sensitive to the small amount present or the linkage is such as to give negative results. It does, however, lead one to interpret the results with Millon's test to indicate the presence of oxyacids.

In the one-day cultures of *Azotobacter* and *Rhizobium* we find measur-



TABLE IV  
QUALITATIVE PROTEIN TESTS ON CULTURES OF RHIZOBIUM AND AZOTOBACTER

|   | Biuret | Hopkins-Gole   | Bromine | Millon's | Xantho<br>proteic | Morner's |
|---|--------|--|---------|----------|-------------------|----------|
|   |        | (Cultures of Rhizobium in Moore's Solution)                                    |         |          |                   |          |
| 2 days  | —      | —  | —       | +        | —                 | —        |
| 4 days  | —      | —  | —       | +        | —                 | —        |
| 8 days  | —      | —  | —       | +        | +                 | —        |
|   |        | (Cultures of Rhizobium grown in Dextrose-Ashby's Solution)                     |         |          |                   |          |
| 1 day   | +      | —  | —       | —        | —                 | —        |
| 2 days  | +      | —  | —       | +        | —                 | —        |
| 4 days  | +      | —  | —       | +        | —                 | —        |
| 8 days  | +      | —  | —       | +        | —                 | —        |
|   |        | (Cultures of Azotobacter grown in Dextrose-Ashby's Solution)                   |         |          |                   |          |
| 1 day   | +      | —  | —       | —        | +                 | —        |
| 2 days  | +      | —  | —       | +        | +                 | —        |
| 4 days  | +      | —  | —       | +        | +                 | —        |
| 8 days  | +      | —  | —       | +        | +                 | —        |
|   |        | (Azotobacter and Rhizobium cultures 2½ years old in Dextrose-Ashby's Solution) |         |          |                   |          |
| 1. Rhizobium<br>(No Lime)   | +      | —  | —       | +        | +                 | —        |
| 2. Rhizobium<br>(Excess of Lime)                                  | +      | —  | —       | +        | —                 | —        |
| 3. Azotobacter<br>(No Lime)                                       | +      | +  | —       | +        | +                 | —        |
| 4. Azotobacter<br>(Excess of Lime)                                | +      | +  | —       | +        | +                 | —        |
| 5. Check  | —      | —  | —       | —        | —                 | —        |
| 6. Rhizobium<br>(Washed off fresh<br>Cultures<br>on Moore's agar) | +      | —  | —       | +        | —                 | —        |

able quantities of ammonia where Millon's reaction is negative. This might well be taken to indicate the course of synthesis as passing through the ammonia state, the ammonia possibly replacing an OH group of an oxyacid, thus forming an amino acid. Since Stoklasa (20) has demonstrated the production of hydrogen by *Azotobacter* it is possible that in this way a molecule of water may be formed. The amino acid would be subsequently synthesized to protein.

Since amino acids and ammonia are present in cultures in which no nitrates or nitrites are found, we naturally ask, "Is ammonia the first product formed in the fixation of atmospheric nitrogen by nitrogen-fixing bacteria?" To answer this question twelve 500 c.c. portions of modified Ashby's solution having a reaction of pH 7.8 but containing no lime, were placed in one-liter flasks equipped with aeration tubes and sterilized. These were connected with receiving flasks containing N/50 HCl, also fitted with aeration connections. Cotton filters and flasks containing 20 percent sulphuric acid were used to take out any ammonia or bacteria from the air that was drawn through the medium by means of a suction pump. Four flasks were inoculated with *Azotobacter chroococcum*, four with *Rhizobium leguminosarum* and four were not inoculated. After one week one flask of *Azotobacter*, one of *Rhizobium* and one blank were removed for analysis. This was repeated at the end of two weeks, four weeks, and eight weeks.

Ammonia from the cultures of *Azotobacter* and *Rhizobium* was carried off by air current and collected in receiving flasks. The amount collected, however, was small, as at the end of one week the *Azotobacter* stopped growing and at the end of four weeks the *Rhizobium* had apparently stopped growing. The following Table V shows the mgs. of nitrogen carried over as ammonia at the end of each period of aeration. The growth of organisms was not luxuriant and the amount of nitrogen fixed was small.

To check further on the formation of ammonia three-liter portions of modified Ashby's solution containing 5 grams of calcium carbonate per liter were placed in four-liter flasks equipped with aeration tubes, were sterilized and connected in series with receiving flasks containing N/50 HCl fitted with aeration connections. A strong current of air which had

TABLE V  
MGS. OF NITROGEN AS AMMONIA AERATED FROM CULTURES  
CONTAINING NO LIME

|                    | 1 week | 2 weeks | 3 weeks | 8 weeks |
|--------------------|--------|---------|---------|---------|
| <i>Azotobacter</i> | .37    | .37     | .37     | .25     |
| <i>Rhizobium</i>   | none   | .13     | .53     | .36     |
| Blank              | none   | none    | none    | none    |

been filtered through 20 percent sulphuric acid and cotton kept the solutions vigorously agitated. One flask was inoculated with *Azotobacter chroococcum*, one with *Rhizobium leguminosarum* (alfalfa strain) while one was left blank. At one-week intervals for four weeks the receiving flasks were titrated, but no ammonia had been carried over by the current of air. The organisms grew luxuriantly and at the end of two weeks the cultures of *Azotobacter* and *Rhizobium* had become very viscid. The *Azotobacter* culture became characteristically amber colored, while the *Rhizo-*

*bium* culture assumed the characteristic mucilaginous appearance. Analyses of the cultures after the four-week aeration gave results as follows: (Table VI)

The failure of these cultures to give off ammonia in the current of air as in the previous test, where no lime was used, may be due to the union between the ammonia formed and the calcium bicarbonate, the calcium bicarbonate being formed through the action of carbon dioxide on the calcium carbonate. One very surprising thing is the large amount of nitrogen fixed by the *Rhizobium* culture. This brings out the possibility that some condition must exist in these cultures whereby the ammonia is withheld

TABLE VI  
ANALYSES OF AERATED CULTURES CONTAINING LIME

|             | Total N per<br>100 c.c.<br>of culture | Amino N*<br>per 100 c.c.<br>of culture | Ammonia N<br>per 100 c.c.<br>of culture | Nitrates | Nitrites |
|-------------|---------------------------------------|--|---|----------|----------|
| Azotobacter | 8.6 mg.                               | .56 mg.                                | .03                                     | None     | None     |
| Rhizobium   | 7.2                                   | None                                   | .03                                     | None     | None     |
| Blank       | None                                  | None                                   | None                                    | None     | None     |

\* The formaldehyde was allowed to stand in contact with the culture for two days before being titrated.

from the cell, somewhat as happens within the nodule when the plant removes the soluble nitrogen formed causing the organisms to fix large quantities of nitrogen in order to have enough available for the bacterial cell.

The hydrogen-ion concentration of this medium was pH 7.6 according to colorimetric determinations made on all the flasks before inoculating and after four weeks' incubation.

#### Discussion

The data presented, although dealing with such small quantities of nitrogen as to greatly lessen the value and reliability of the recognized quantitative methods, indicate that in the process of nitrogen fixation ammonia appears in measurable amounts in rapidly growing cultures before sufficient amino or protein nitrogen has accumulated to be demonstrated by the well-recognized qualitative tests. The ammonia content diminishes as the culture grows even though the amount of nitrogen fixed is several times greater than it was at the earlier period. Another interesting fact is that in the presence of lime a vigorously growing, aerated culture of *Rhizobium* will fix comparatively large quantities of nitrogen, but no ammonia is given off. Without the lime a small amount of ammonia is given off, but little nitrogen is fixed.

The amino nitrogen is always low in young cultures compared to the total nitrogen possibly due to the rapidity with which it is synthesized into protein. In the old cultures the amino content is much higher. This may be interpreted to mean that autolysis has taken place or it may mean that synthesis is slow.

It is significant that in general the qualitative protein tests are the same for both old and young cultures. One exception is the Hopkins-Cole

reaction on the old *Azotobacter* cultures. Tyrosine or oxyacids are evidently formed in the protein molecule during the process of synthesis.

Nitrates and nitrites are apparently not formed in the process of nitrogen fixation by either of the organisms studied.

#### Conclusions

This investigation has led to the following conclusions:

1. A vigorous growth of legume bacteria and *Azotobacter* can be effected and enough combined nitrogen formed in a few days to permit the use of analytical chemical methods for detecting and partitioning the various forms.

2. A two percent solution of sucrose is poorly adapted to this type of work because of heavy gum production in this medium.

3. The formaldehyde (Sorenson) titration method is quite unreliable when used in connection with bacterial cells because the formaldehyde must remain in contact with the cells for some time before the reaction is completed. Van Slyke's method is better suited to this purpose, though in young cultures the amount of amino nitrogen in synthetic media is so small as to be within the experimental error of this method where a maximum of ten cubic centimeters of culture solution can be used in one determination. This fact makes the Sorenson formaldehyde method valuable in dealing with these small quantities of amino nitrogen by employing large quantities of the material in the titration.

4. Qualitative tests indicate the presence of tyrosine or oxyacids in all the cultures studied.

5. The amount of amino nitrogen increases with the age of the culture up to a certain point.

6. Nitrates and nitrites could not be detected at any time in vigorous growing cultures when the reagents used in making up the media were free from these compounds.

7. The fact that an accumulation of ammonia always occurs in the cultures during the first few days of maximum growth and gradually diminishes as the source of carbohydrate is exhausted leads to the conclusion that ammonia is probably the first product of metabolism in nitrogen fixation and that soon a balance is reached within the cells in which the ammonia is formed no faster than it is synthesized into protein. No attempts have been made to prove the possibility of the production of nascent hydrogen to unite with the free nitrogen of the air.

8. Small amounts of ammonia are carried off from cultures of *Rhizobium leguminosarum* and *Azotobacter chroococcum* growing in dextrose Ashby's solution containing no lime if the cultures are aerated vigorously during the first week's growth.

9. When an excess of lime is added to dextrose Ashby's solution no ammonia is liberated by the aeration method. Under these conditions the *Rhizobium* culture fixed an unusually large amount of nitrogen and analyses demonstrated the presence of ammonia.

10. *Rhizobium leguminosarum* grown in vigorously aerated dextrose-Ashby's solution fixes relatively large quantities of atmospheric nitrogen.

11. From the data accumulated, the conclusion seems justified that the first products of synthesis are the same for *Rhizobium leguminosarum* and *Azotobacter chroococcum*.

12. Soluble forms of nitrogen are formed by nitrogen-fixing bacteria

during the early stages of growth when these bacteria are grown independent of higher plants or their enzymes.

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# THE PRODUCTION OF A YEAST GROWTH STIMULANT BY HEATING MEDIA UNDER PRESSURE

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During the course of work with synthetic media for the growth of yeast in this laboratory unusually large growths were occasionally experienced. In each instance this anomalous growth took place in a medium which had been caramelized during the process of sterilization. But while the increased growth was invariably associated with caramelization of the medium, a caramelized medium did not always show the stimulating effect.

In this paper are recorded the results of a study of the effect of sterilization of several media under pressure upon their ability to support the growth of yeast.

## I. EXPERIMENTAL TECHNIQUE AND EQUIPMENT

The yeast used was a culture of *Saccharomyces cerevisiae*. The yeast had been growing in synthetic media for a number of years with an occasional plating on the synthetic agar medium of Fulmer and Grimes (1923).

The following media were used, as developed by Fulmer, Nelson and Sherwood (1921). The concentrations are in grams per 100 cubic centimeters of medium.

TABLE I  
MEDIA USED

|                                 | C     | D     | E     | F     |
|---------------------------------|-------|-------|-------|-------|
| NH <sub>4</sub> Cl              | 0.188 | 0.188 | 0.188 | 0.188 |
| K <sub>2</sub> HPO <sub>4</sub> | 0.100 | 0.100 | 0.100 | 0.100 |
| CaCl <sub>2</sub>               |       | 0.100 | 0.100 | 0.100 |
| CaCO <sub>3</sub>               |       |       | 0.040 | 0.040 |
| Dextrin                         |       |       |       | 0.60  |
| Cane Sugar                      | 10.0  | 10.0  | 10.0  | 10.0  |

The inoculation was made by the addition of 1 cubic centimeter of yeast emulsion to 19 cubic centimeters of the medium, hence the medium was made up to a strength of 20/19 of the above in order to take care of dilution.

The number of cells was determined by means of the Thoma-Zeiss counting chamber. The concentration of yeast is expressed as "Count," or the number of cells in sixteen small squares. When the count equals 1, there are 250,000 cells per cubic centimeter. In all cases the inoculation was such as to make the initial count equal to one. The inoculation was always made from an actively growing culture. All incubations were made at 30° C.

In determining pH a standard potentiometric set up was used with a saturated KCl calomel half cell and, at first, a Bailey hydrogen electrode.

In later experiments the quinhydrone electrode was used as described by Biilmann (1921) and Kolthoff and Furman (1926).

## II. EXPERIMENTAL RESULTS

### A. Media C, D, E, and F sterilized for different lengths of time.

Media C, D, E, and F were sterilized under 15 pounds pressure for various lengths of time, inoculated, incubated for 48 hours, and the counts made. The results are given in Table II.

TABLE II

EFFECT OF TIME OF STERILIZATION OF MEDIA UPON GROWTH OF YEAST

| Time of Sterilization<br>(Minutes) | C   | D   | E  | F  |
|------------------------------------|-----|-----|----|----|
| 15                                 | 19  | 51  | 52 | 50 |
| 30                                 | 32  | 60  | 51 | 37 |
| 45                                 | 39  | 90x | 43 | 41 |
| 60                                 | 46x | 66  | 50 | 24 |
| 75                                 | 25  | 70  | 48 | 33 |
| Order of Color<br>x Optimum.       | 4   | 3   | 2  | 1  |

An examination of the above data brings out some interesting points. The growth in Medium C is nearly doubled when the period of sterilization is extended from 15 to 30 minutes. There is a maximum growth for the 60 minute period sterilization. The optimum for Medium D is at 45 minutes. There is no stimulation in Mediums E and F, in fact in the latter medium there seems to be a toxic effect. In the last line of Table II is given the order of color of the media; i.e., degree of caramelization. It might seem that failure to obtain stimulation in Medium E is due to the fact that the optimum has been passed. This idea seems to be negated by the fact that the color in Medium E at 30 minutes is not greater than in Medium C at 60 minutes.

Since the degree of stimulation in Medium C is greater than in any other medium and furthermore since this medium is the least complex, it was decided to use only this medium for further study.

### B. The effect of various "caramel" preparations upon the growth of yeast.

The fact that only a caramelized medium shows the yeast growth stimulation does not prove that caramel is the stimulant rather than some other product or products produced simultaneously with the caramel bodies. Hence the terminology "caramel" or caramel preparation to designate the product used in the experiments.

In order to standardize the procedure, the following arbitrary method was adopted to test the effect of various concentrations of "caramel" upon the growth of yeast. Five hundred cubic centimeters of the medium were sterilized for the desired interval and this preparation designated as 100%. These preparations were then added in various ratios to the uncaramelized medium. Thus 5% means that 5 cubic centimeters of the preparation were used to 95 cubic centimeters of the uncaramelized medium. The mixture was then sterilized for 15 minutes in live steam.

In Table III are given data for the growth of yeast in Medium C con-

largely active acid. The data on this culture were accordingly checked and then, since there was the possibility of the culture being impure, it was plated out on whey agar, colonies picked into litmus milk, and two of the resulting cultures (A3A and A3B) studied as to the type of lactic acid produced. The data secured with A3 and the cultures derived from it are given in Table III.

From the results presented it is evident that with culture A3 in both trials and with the two cultures secured from it by plating, the type of lactic acid produced was quite uniform. The variations in the percent  $H_2O$  of crystallization are surprisingly small when consideration is given to the fact that the completeness of crystallization of the zinc lactate influences this percentage when there is a mixture of active and inactive acids. The percent  $ZnO$  in all of the lactates approaches the theoretical very closely. It accordingly appears that with this culture, at least, the production of considerable inactive acid is a definite character of the organism.

Table IV compares the data obtained on the zinc lactates prepared by the wet extraction method with those prepared by the dry extraction method, where equal amounts of whey from the same lot were used for each comparison. The results representing the dry extraction method have already been given in the preceding tables but are here repeated so that the comparisons can more easily be made. There is a general agreement in the values obtained on the two preparations of zinc lactate from the same whey—at least the agreement is such that either method would apparently give a satisfactory idea of the type of lactic acid produced. The largest difference in the moisture contents of the lactates secured by the two methods was with culture S6 where the value with the dry method was 13.45 and with the wet method 15.235%. In the instances where there was

TABLE III  
RESULTS OF CHECKING THE TYPE OF LACTIC ACID PRODUCED  
BY CULTURE A3

| Cultur<br>No. | Results on zinc lactates            |       |       |        |        |                  | Rota-<br>tion |
|---------------|-------------------------------------|-------|-------|--------|--------|------------------|---------------|
|               | H <sub>2</sub> O of crystallization |       |       | det. A | det. B | av.              |               |
|               | ZnO                                 |       |       |        |        |                  |               |
| A3            | first trial—from table 1            | 16.89 | 17.10 | 16.995 | 33.74  | very<br>slight 1 |               |
| A3            | second trial                        | 15.11 | 15.62 | 15.365 | 33.67  | very<br>slight 1 |               |
| A3A           | secured by replating A3             | 16.94 | 17.08 | 17.01  | 33.67  | very<br>slight 1 |               |
| A3B           | secured by replating A3             | 17.49 | 17.69 | 17.59  | 33.67  | very<br>slight 1 |               |

an appreciable difference in the moisture content of the salts obtained by the two methods the dry method gave the lower value, which presumably means that the acid contained a larger proportion of the active form. The duplicate determinations of the  $H_2O$  of crystallization agree better with the dry extraction preparations than with the wet extraction salts; this may be due to the larger amounts of material that were available following dry extraction. The  $ZnO$  percentages agree reasonably well with the theoretical, although an occasional value is rather high.

TABLE II  
CHARACTERS OF THE STRAINS OF *L. ACIDOPHILUS* STUDIED

| Culture no. | Acid from maltose | Growth at surface tension of |            | Growth in milk |          | Acidity* in milk held 7 days at 37°C. | Comparative size |
|-------------|-------------------|------------------------------|------------|----------------|----------|---------------------------------------|------------------|
|             |                   | 40 dynes                     | 37.4 dynes | at 15-20°C.    | at 45°C. |                                       |                  |
| A1          | +                 | +                            | +          | +              | —        | 1.34%                                 | small            |
| A3          | +                 | +                            | —          | —              | +        | 2.02                                  | large            |
| A4          | +                 | +                            | —          | —              | +        | 1.37                                  | large            |
| A5          | +                 | +                            | —          | —              | +        | 1.59                                  | large            |
| A6          | —                 | +                            | —          | —              | +        | 1.17                                  | large            |
| S1          | +                 | +                            | +          | +              | —        | 1.26                                  | small            |
| S5          | +                 | +                            | —          | +              | —        | 1.35                                  | small            |
| S6          | +                 | +                            | +          | +              | —        | 1.07                                  | small            |
| S7          | +                 | +                            | +          | +              | —        | 1.45                                  | small            |
| S8C         | +                 | +                            | +          | +              | —        | 1.85                                  | large            |
| S11         | +                 | +                            | —          | +              | —        | 1.35                                  | large            |
| S12         | +                 | +                            | +          | +              | —        | 1.70                                  | small            |

\* Calculated as lactic acid.



TABLE I  
RESULTS ON ZINC LACTATES PREPARED FROM MILK FERMENTED BY  
*L. ACIDOPHILUS* CULTURES

| Culture No. | Source of culture                   |  |        |        | Rotation | pH     |
|-------------|-------------------------------------|--|--------|--------|----------|--------|
|             | H <sub>2</sub> O of crystallization |  | det. A | det. B |          |        |
|             | av.                                 |  | %      | %      | %        |        |
| A1          |                                     |  | 12.85  | 12.96  | 12.905   | 33.64  |
|             |                                     |  |        |        |          | 1      |
| A3          |                                     |  | 16.89  | 17.10  | 16.995   | 33.74  |
|             |                                     |  |        |        |          | slight |
| A4          |                                     |  | 18.06  | 18.09  | 18.075   | 33.92  |
| A5          |                                     |  | 16.43  | 16.49  | 16.46    | 34.13  |
| A6          |                                     |  | 18.15  | 18.15  | 18.15    |        |
| S1          |                                     |  | 12.71  | 12.90  | 12.805   | 33.74  |
| S5          |                                     |  | 12.88  | 12.71  | 12.795   | 33.50  |
| S6          |                                     |  | 13.41  | 13.49  | 13.45    | 33.78  |
| S7          |                                     |  | 13.78  | 13.50  | 13.64    | 34.50  |
| S8C         |                                     |  | 14.04  | 14.10  | 14.07    | 33.78  |
| S11         |                                     |  | 12.95  | 12.99  | 12.97    | 33.52  |
| S12*        |                                     |  | 14.34  | 14.28  | 14.31    | 35.49  |
|             |                                     |  |        |        |          | 1      |

\* By wet extraction.

anhydrous salt). In some instances the amount of salt available for this determination was rather small for highly accurate results. With the present uncertainty as to the classification of *L. acidophilus* and the closely related types, the characters which at present appear to be of the most significance are presented in Table II. The data given show that one of the 12 cultures did not produce acid from maltose, although *L. acidophilus* is said to ferment this sugar. The medium used for testing maltose fermentation was medium X of Albus and Holm (1926) with maltose replacing the lactose. All of the cultures grew at a surface tension of 40 dynes but only six at 37.4 dynes; for the surface tension trials medium X of Albus and Holm was used and sodium ricinoleate was the depressant added. Four of the six organisms that failed to grow at the lower surface tension where among the five secured from other laboratories. Only four of the 12 organisms grew at 45°C. and these were also the only ones which failed to grow at from 15-20°C.; they were the same four from other laboratories which failed to grow at the lower surface tension. The total acidities produced in milk by the organisms growing for 7 days at 37°C. varied from 1.07 to 2.02%. While the highest acidity was produced by one of the organisms of the group of four showing a relationship in characters, other organisms of this group produced comparatively low total acidities. There was considerable variation in the size of the individual cells with the twelve cultures investigated; on a basis of only two groups (large and small) six were classed as large and six as small. The four cultures which grew at 45°C. but not at 15-20°C. and which did not develop at the lower surface tension were all comparatively large. It should be noted that these four cultures were the ones which produced largely or entirely inactive lactic acid. One of these cultures, however, was the only one of the twelve which failed to ferment maltose.

Culture A3 was one of the first cultures studied that did not produce

dry to practically constant weight. For the wet extraction 600 c.c. of whey, prepared as outlined above, were extracted in a Kutscher and Stendal apparatus. The more common extraction period was 48 hours but in two runs a 72 hour period was used. The ether and dissolved materials were purified, and recovered in the same manner as when the dry extraction method was employed.

Twelve cultures of *L. acidophilus* were studied as to the isomeric form of lactic acid produced in milk; seven of these were isolated by the writers and the other five came from five different laboratories, four commercial and one research. The data secured on the zinc lactates are given in Table I together with the sources of the organisms.

From these results it is evident that among the *L. acidophilus* cultures studied the type of lactic acid formed was not uniform but varied from pure active to practically pure inactive with various mixtures of these. Where there was active acid it was of the *d* form.

## Results

amounts of laevo and dextro acids by *B. bulgaricus* from glucose, galactose, levulose, and mannose but for some unexplained reason a relative excess of the latter in milk.

White and Avery (1910) found their high acid type (Type A) from yoghurt, mazun, and leben formed either inactive or laevo acid, while their low acid type (Type B) formed principally laevo, more rarely inactive or dextro.

Varying results were obtained by Currie (1911) who found that high acid strains of *B. bulgaricus* from various sources formed an excess of dextro acid. Two cultures from milk soured at 38°C. formed inactive and laevo; two from cheddar cheese formed dextro and inactive; two from cheddar cheese formed inactive and laevo; one from cheddar cheese formed only laevo; of 20 other strains, 13 formed dextro only and seven inactive only. In a study of the lactic acid in cheddar cheese, Hart, Hastings, Flint and Evans (1914) isolated organisms belonging to a group which they called *Bacterium cassi*; one of these produced laevo lactic acid and two dextro. A mixture of the two types gave the racemic acid with a slight excess of laevo.

Heinemann and Becker (1916) determined the form of lactic acid produced in milk by three strains of the Boas-Oppler bacillus from gastric ulcers and found it to be laevo. This organism was considered identical with *B. bulgaricus* by Heinemann and Heffernan (1909) and by Galt and Iles (1915).

Orla-Jensen (1921) reported *Thermobacterium bulgaricum* (*Bacillus bulgaricus*) as producing up to 1.7% of laevo acid.

In a study of 15 cultures of Döderlein's vaginal bacillus and *B. acidophilus*, Jöten (1922) found that all formed inactive lactic acid.

McIntosh, James and Lazarus-Barlow (1924) reported that Dadds found *B. acidophilus-odontolyticus*, which some investigators consider identical with *L. acidophilus*, produced malic acid with only a trace of lactic.

Pederson, Peterson, and Fred (1926) found that a sample of acidophilus milk contained a slight excess of laevo acid. The milk had not been sterilized before inoculation so the results are comparable to those found with naturally soured milks.

Kopeloff (1926) stated that the unpublished results of Zoller show that "the non-volatile acid of acidophilus is found to be entirely dextro lactic acid." Zoller further gave as his opinion that the stereomorphism of the lactic acid constituted one point of differentiation between *L. acidophilus* and *L. bulgaricus*. One culture of *L. bulgaricus* produced laevo lactic acid.

### Methods

The organisms studied were grown in flasks of milk held at 37°C. for one week. The zinc lactates were then prepared as follows:  
*Dry Extraction Method.* The fermented milk was heated by standing the flask containing it in hot water to facilitate the separation of the whey. Usually about 5 c.c. of N/1  $H_2SO_4$  were added per 100 c.c. of milk before heating to help free the lactic acid, but in some runs no  $H_2SO_4$  was used. The heated milk was thrown on a filter paper and the whey collected. The whey was then heated in an evaporating dish on a water bath until only a

# THE ISOMERIC FORM OF LACTIC ACID PRODUCED BY LACTOBACILLUS ACIDOPHILUS

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Among the organisms belonging to the genus *Lactobacillus* there are three—*L. casei*, *L. acidophilus* and *L. bulgaricus*—which are of considerable importance from the standpoint of the dairy industry. *L. casei* apparently plays a necessary role in the ripening of various types of hard cheeses while *L. acidophilus* and *L. bulgaricus* are employed in the preparation of fermented milk intended to influence the bacterial flora of the intestinal tract; *L. bulgaricus* also seems to be of use in the Swiss cheese industry. These three organisms have various characters in common and are not easily distinguished, indeed certain data even indicate that there may not be three distinct species. Various procedures have been suggested for the separation of these organisms, among them sugar fermentations, suitability of media with different surface tensions, and temperatures at which growth occurs.

In connection with a study of certain cultures of *L. acidophilus*, determinations were made of the isomeric form of lactic acid produced in milk, since it was thought that such data might be suggestive of the relationship of this organism. The zinc lactates studied were prepared under as uniform conditions as possible so that the results could be directly compared. The usual procedure employed in securing the lactic acid was a dry extraction method but in a few instances a wet extraction method, using whey from the same lot, was compared with this, both as to the form of lactic acid and as to the yield.

## Historical

One of the earliest reports on the type of lactic acid produced by the *Lactobacilli* is that of Orla-Jensen (1904) who stated that *B. casei epsilon* may produce over 2.7% of inactive lactic acid in milk. The zinc lactate prepared from the acid contained 18.2% of water of crystallization and 27.5% of  $ZnO$ ; it showed no rotatory properties.

Bertrand and Weisweiler (1906) concluded from a study of cultures of *B. bulgaricus* supplied by Metchnikoff that the lactic acid produced by the organisms was a mixture of the laevo and dextro forms, with the latter predominating. Grigoroff (1905), on the other hand, stated that his *Bacillus A* (*B. bulgaricus*) produced the inactive form. Of less value is the statement of Lueresen and Kühn (1908) that the whey from milk fermented by *B. bulgaricus* turned polarized light to the right.

Heinemann and Heffernan (1909) found the acid produced by *B. bulgaricus* to be inactive, without a trace of the active form being left in the mother liquor after removing the zinc salts.

Bertrand and Duchacek (1909) reported the production of equal





be a caramel or a product or products formed simultaneously with the former.

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(1) Double concentration.

(2) By "Complement,"

(2) By "Complement" is meant the solution containing the salts necessary to make the solution have the same composition as Medium C; thus the Complement (double concentration) to allow for adjusting the pH and for inoculation) for "A" would contain per 100 cubic centimeters of  $\text{H}_2\text{O}$ , 20 grams of sugar, 0.400 grams of  $\text{K}_2\text{HPO}_4$ , and no  $\text{NH}_4\text{Cl}$ .

By "a," is meant the number of cubic centimeters of acid or alkali needed to adjust the solution to a pH of 6.24. These solutions were then inoculated and the counts at the end of 19

hours are given in Table IX.

From these results it is seen that Caramel "A", up to 50% gives no optimum. Further addition gives an increased count. "B" gives an optimum at 20% and the addition of more of this preparation then decreases the count. The counts of the 50% "A" and 20% "B" are nearly identical.

Since these two preparations give such very different results from each other and from the ("C") Caramel, it is of interest to know whether ("C") is a combination of equal parts of ("A") and ("B"), or a different product which is formed when the two are produced in the same solution. For this purpose solutions containing various percentages of a 1:1 combination of ("A," plus "B,") (a 10% solution—(total volume 20 cubic centimeters) would contain 1 cubic centimeter of each, thus containing 2 cubic centimeters or 10% of the mixture) diluted with Medium C were prepared, sterilized, adjusted to a pH of 6.24, inoculated, and counted after 19 hours. The results are given in Table IX.

TABLE IX  
RELATIVE GROWTH IN SOLUTIONS OF DIFFERENT CARAMELS

| Caramel |                | A     |                | B     |           | A Plus B |           |
|---------|----------------|-------|----------------|-------|-----------|----------|-----------|
| Count   | % Caramel      | Count | % Caramel      | Count | % Caramel | Count    | % Caramel |
| I       | 13             | I     | 13             | I     | 13        | I        | 13        |
| II      | 19             | II    | 19             | II    | 22        | II       | 19        |
|         | 33             |       | 30             |       | 26        |          | 29        |
|         | 37             |       | 32             |       | 27        |          | 32        |
| 41x     | 36x            |       | 41             |       | 36        |          | 29        |
|         | 34             |       | 37             |       | 35        |          | 34        |
|         | 33             |       | 44             |       | 36        |          | 37        |
|         | 50-(           |       | 41             |       | 36        |          | 30        |
|         | 40-(           |       | 35             |       | 35        |          | 34        |
|         | 30-(           |       | 26             |       | 26        |          | 29        |
|         | 20-(           |       | 13             |       | 13        |          | 28        |
|         | 10-(           |       | 10             |       | 10        |          | 33        |
|         | 0-(unadjusted) |       | 13             |       | 13        |          | 22        |
|         | 0-(pH = 6.24)  |       | 10             |       | 10        |          | 10        |
|         |                |       | 13             |       | 13        |          | 13        |
|         |                |       | 19             |       | 19        |          | 13        |
|         |                |       | 22             |       | 22        |          | 10        |
|         |                |       | 33             |       | 26        |          | 22        |
|         |                |       | 37             |       | 27        |          | 19        |
|         |                |       | 36x            |       | 36        |          | 10        |
|         |                |       | 33             |       | 35        |          | 13        |
|         |                |       | 37             |       | 36        |          | 19        |
|         |                |       | 44             |       | 35        |          | 22        |
|         |                |       | 32             |       | 36        |          | 26        |
|         |                |       | 33             |       | 35        |          | 27        |
|         |                |       | 34             |       | 36        |          | 28        |
|         |                |       | 37             |       | 37        |          | 30        |
|         |                |       | 41             |       | 38        |          | 31        |
|         |                |       | 50-(           |       | 41x       |          | 33        |
|         |                |       | 40-(           |       | 36x       |          | 34        |
|         |                |       | 30-(           |       | 33        |          | 37        |
|         |                |       | 20-(           |       | 32        |          | 41x       |
|         |                |       | 10-(           |       | 27        |          | 41x       |
|         |                |       | 0-(unadjusted) |       | 26        |          | 31        |
|         |                |       | 0-(pH = 6.24)  |       | 19        |          | 30        |
|         |                |       |                |       | 13        |          | 37        |
|         |                |       |                |       | 10        |          | 34        |
|         |                |       |                |       | 13        |          | 37        |
|         |                |       |                |       | 19        |          | 37        |
|         |                |       |                |       | 22        |          | 37        |
|         |                |       |                |       | 26        |          | 37        |
|         |                |       |                |       | 27        |          | 37        |
|         |                |       |                |       | 36        |          | 37        |
|         |                |       |                |       | 35        |          | 37        |
|         |                |       |                |       | 36        |          | 37        |
|         |                |       |                |       | 37        |          | 37        |
|         |                |       |                |       | 41        |          | 37        |
|         |                |       |                |       | 44        |          | 37        |
|         |                |       |                |       | 50-(      |          | 37        |

From these results it is seen that "C," is not a combination of equal parts of "A" and "B," since "C," shows an optimum at 30%, whereas the addition of the combination of "A" plus "B," causes a steady increase in the count, somewhat similar to the effect produced by "A," alone.

## SUMMARY

The sterilization of several media under pressure has been shown to produce a yeast growth stimulant. Stimulating factors were likewise produced by the heating of sugar solution under pressure with ammonium chloride, dipotassium phosphate or a mixture of the two salts. The stimulation is not due to a change in the pH of the media. The stimulant may

a constant pH. Such a series, all at a pH of about 7, was made up, inoculated, and incubated for 48 hours. The results are shown in Table VII.

TABLE VII

GROWTH IN DIFFERENT % SOLUTIONS OF CARAMEL AT A pH OF 7

| Count | % Caramel |       | pH   |       | I    |      | II    |      |
|-------|-----------|-------|------|-------|------|------|-------|------|
|       |           |       |      |       |      |      |       |      |
| 0     | 6.98      | 25.8  | 7.11 | 33.1  | 31.3 | 31.3 | 31.3  | 31.3 |
| 10    | 7.11      | 33.1  | 7.11 | 33.1  | 32.0 | 32.0 | 31.5  | 32.0 |
| 20    | 7.11      | 33.1  | 7.11 | 33.1  | 31.5 | 31.5 | 33.8x | 31.5 |
| 30    | 7.11      | 36.0x | 7.11 | 36.0x | 34.5 | 34.5 | 30.6  | 34.5 |
| 40    | 7.05      | 34.5  | 7.05 | 34.5  | 27.4 | 27.4 |       |      |
| 50    | 7.05      | 27.4  | 7.05 | 27.4  |      |      |       |      |

While the data show an optimum at 30%, it is not as pronounced as in the previous results. This would indicate that a pH of about 7 is so

detrimental as to partly overshadow the optimum. If the latter is the case, any such harmful effect would be done away with by adjusting the pH

to 6.24. This was done, the flasks inoculated and incubated for 19 hours. The counts are given in Table IX, Column for Caramel C.

These counts show a distinct optimum at 30% which must be due to something within the caramelize medium, since we have shown that it is

not a function of pH alone.

D. Caramels A and B, and their effect upon the growth of yeast in

Medium C.

In the above described experiments the caramelize was produced by a mixture of the two salts,  $K_2HPO_4$  and  $NH_4Cl$ . Experiments were

next undertaken to determine the role of each of these salts in the production of the stimulant. Solutions containing sugar and  $NH_4Cl$  and sugar

and  $K_2HPO_4$  were caramelize and designated as caramels "A" and "B" respectively. To the preparation used heretofore, containing  $NH_4Cl$  and

$K_2HPO_4$ , was given the name Caramel "C."

"A," "B," and "C" are easily distinguished from one another. "A" is a brown solution which becomes cloudy upon standing a day or more;

"B" is a brownish yellow and remains clear indefinitely; while "C" is nearly

decolorized by the addition of hydrochloric acid, while "A" is "acid fast."

All give a positive test with Fehling's solution. The respective pH's are:

"A," 4.0; "B," 6.8; and "C," 6.0.

Solutions containing varying concentrations of each of these caramels

were made up and adjusted to a pH of 6.24. Table VIII shows the amounts

of solutions used in making up such a series.

TABLE VIII

COMPOSITION OF PERCENTAGE SOLUTIONS OF "A" AND "B"

| % Caramel | Medium C | Complement | Caramel | $H_2O$ |
|-----------|----------|------------|---------|--------|
|           |          |            |         |        |
| 10        | 8        | 1          | 2       | 8-a    |
| 20        | 6        | 2          | 4       | 7-a    |
| 30        | 4        | 3          | 6       | 6-a    |
| 40        | 2        | 4          | 8       | 5-a    |
| 50        | 0        | 5          | 10      | 4-a    |

It should be noted that the products produced during caramelizeation lead to the increased production of acid during sterilization. Hence in a series of media containing various concentrations of "caramel" not only would the pH change during sterilization but the change would be greater the higher the concentration of the "caramel."

In the succeeding experiments the pH was adjusted after sterilization by the method described by Christensen and Fulmer (1925). In Table V are given data showing the effect of pH upon the growth of yeast in Medium C.

TABLE V  
INFLUENCE OF pH (AFTER STERILIZATION) UPON GROWTH OF  
YEAST IN MEDIUM C

| Counts in Media |    | pH   |
|-----------------|----|------|
| I               | II |      |
| 3               | 2  | 3.21 |
| 15              | 16 | 5.32 |
| 20              | 25 | 6.10 |
| 27              | 31 | 6.48 |
| 9               | 9  | 6.87 |
| 5               | 5  | 7.99 |
| 4               | 7  | 8.19 |
| 2               | 2  | 8.43 |

From the above it will be seen that the optimum pH (taken after sterilization) for the growth of yeast in Medium C is between 6.10 and 6.48. The pH of the optimum concentration of the caramel preparation (30%) is 6.24. Since this is also the optimum pH for the growth of yeast in this medium, the next step will be to determine how much of the stimulation is due to the pH. For this purpose Medium C was adjusted to the same pH as the 30% solution of the caramel preparation and flasks of this solution, flasks of Medium C unadjusted, and flasks of the 30% solution of the caramel preparation were inoculated to a count of one. After incubation for 24 hours the counts (Table VIII) show a decided increase in growth in the medium containing the caramel over that in Medium C at the same pH as that of the caramel solution, while the count of the medium adjusted to pH=6.24 shows much better than that of the medium which is unadjusted.

TABLE VI  
COMPARISON OF GROWTH IN 30% CARAMEL AND MEDIUM C

| Count |    | pH   | Medium C | 30% Caramel |
|-------|----|------|----------|-------------|
| I     | II |      |          |             |
| 6     | 6  | 6.82 | Medium C | Medium C    |
| 20    | 19 | 6.24 | Medium C | 30% Caramel |
| 34    | 30 | 6.24 |          |             |
| 20    | 20 |      |          |             |
| III   |    |      |          |             |

Evidently there is some stimulating factor in the medium beside the pH, and this stimulation must come from the caramelizeation because the caramelizeed medium gives an increase in count over the unadjusted medium at a given pH. The exact role of pH in the stimulating effect can be determined only by the use of a series of concentrations of caramel at

taining various percentages of the 3 hour and 5 hour preparations. The columns A B and A', B', C', represent separate experiments. A and A' were counted after the same time interval while B, B', and C' after different periods of growth.

TABLE III

EFFECT UPON THE GROWTH OF YEAST OF VARIOUS PERCENTAGES OF 3-HOUR AND 5-HOUR CARAMEL PREPARATIONS

Count  
 % Caramel Preparation  
 A 3 Hr., "Caramel"  
 B 5 Hr., "Caramel"  
 A' 3 Hr., "Caramel"  
 B' 5 Hr., "Caramel"  
 C

|     |      |      |     |     |     |     |
|-----|------|------|-----|-----|-----|-----|
| 0   | 23   | 51   | 113 | 23  | 64  | 63  |
| 5   | 49x  | 142x | 37  | 60  | 64  | 68  |
| 10  | 47   | 109  | 44  | 57  | 64  | 74  |
| 15  | 37   | 94   | 41  | 59  | 74  | 74  |
| 20  | 39   | 88   | 47  | 65  | 74  | 74  |
| 25  | 39   | 88   | 52x | 67x | 75x | 75x |
| 30  | 41   | 88   | 50  | 49  | 69  | 69  |
| 35  | 43   | 78   | 48  | 50  | 69  | 69  |
| 40  | 41   | 69   | 47  | 45  | 61  | 61  |
| 45  | 62   | 65   | 46  | 52  | 61  | 61  |
| 50  | 64   | 64   | 44  | 38  |     |     |
| 55  | 81   | 81   | 44  | 44  |     |     |
| 60  | 80   | 80   | 43  | 45  |     |     |
| 65  | 90   | 90   | 45  | 49  |     |     |
| 70  | 90   | 90   | 49  | 52  |     |     |
| 75  | 87   | 83   | 42  | 68x |     |     |
| 80  | 134x | 94   | 41  |     |     |     |
| 85  |      |      |     |     |     |     |
| 90  |      |      |     |     |     |     |
| 95  |      |      |     |     |     |     |
| 100 |      |      |     |     |     |     |

x Optimum, see also Tables VII and IX.

The data show that there are two optimum concentrations for each preparation. In the succeeding work the five hour preparation is used as standard and will be designated as "Caramel C."

C. The effect of hydrogen ion concentration upon the growth of yeast in the various media.

Since it is known that yeast growth is a function of pH and the caramel preparation is more acid than Medium C, having a pH of about 6, the next point which presents itself is the possibility of this optimum being a pH effect. The need for the control of this variable is further shown by data in Table IV. The media were all adjusted to a pH=7 before sterilization and then sterilized under 15 pounds pressure for 15 minutes.

TABLE IV

pH CHANGES OF CARAMEL SOLUTIONS DURING 15 MINUTE STERILIZATION

|           |           |          |
|-----------|-----------|----------|
| % Caramel | pH Before | pH After |
| 0         | 7.00      | 6.71     |
| 10        | 7.07      | 6.62     |
| 20        | 7.07      | 6.51     |
| 30        | 7.07      | 6.47     |
| 40        | 7.07      | 6.39     |
| 50        | 7.00      | 6.22     |



TABLE IV  
COMPARISON OF DRY AND WET METHODS OF EXTRACTING LACTIC ACID

| Culture No. | Results on zinc lactates            |            |            |            |               |                                     |            |            |            |          |
|-------------|-------------------------------------|------------|------------|------------|---------------|-------------------------------------|------------|------------|------------|----------|
|             | with dry extraction                 |            |            |            |               | with wet extraction                 |            |            |            |          |
|             | H <sub>2</sub> O of crystallization |            |            |            | Rotation      | H <sub>2</sub> O of crystallization |            |            |            | Rotation |
|             | det. A                              | det. B     | av.        | ZnO        |               | det. A                              | det. B     | av.        | ZnO        |          |
| A3A         | %<br>16.94                          | %<br>17.08 | %<br>17.01 | %<br>33.67 | very slight 1 | %<br>18.17                          | %<br>17.91 | %<br>18.04 | %<br>35.26 |          |
| A4          | 18.06                               | 18.09      | 18.075     | 33.92      |               | 17.75                               | 18.21      | 17.98      | 33.36      |          |
| A5          | 16.43                               | 16.49      | 16.46      | 34.13      |               | 18.06                               | 17.84      | 17.95      | 33.36      |          |
| A6          | 18.15                               |            | 18.15      |            |               | 18.11                               |            | 18.11      |            |          |
| S6          | 13.41                               | 13.49      | 13.45      | 33.78      | 1             | 15.44                               | 15.03      | 15.235     | 33.33      | 1        |
| S7          | 13.78                               | 13.50      | 13.64      | 34.50      | 1             | 13.82                               |            | 13.82      |            |          |
| S8C         | 14.04                               | 14.10      | 14.07      | 33.78      | 1             | 14.30                               | 14.26      | 14.28      | 33.90      | 1        |

Table V gives the weights of the lactates prepared by the two methods of extraction from equivalent amounts of whey; a statement of whether or not  $H_2SO_4$  was added to the milk before filtering off the whey is also included. The yields given indicate the actual weights of the unheated salts that were transferred to dishes on the balance pan and accordingly do not include the amounts lost during grinding and general handling; stated differently, the figures represent the weights of the lactates available for study. In five of the six comparisons the yield was larger with the dry extraction method while with the other the wet method gave a somewhat higher yield. There seemed to be considerable variation in the difference in the yields secured by the two methods, whether figured on an actual weight basis or a percentage basis. The length of time of the extraction by the wet method may have been a factor since in the instance where the wet method gave the higher yield the extraction period was long, but with culture A6 a long extraction period was also used with the wet method and the yield was unusually poor. Whether or not  $H_2SO_4$  had been added to the milk at the time the whey was secured did not seem to be an important factor in determining the yield since both the highest and the lowest yields were secured when the acid was used and without acid the yield was com-

TABLE V  
YIELD OF ZINC LACTATES FROM EQUIVALENT AMOUNTS OF WHEY BY  
THE TWO METHODS OF EXTRACTION

| Culture No. | $H_2SO_4$ added before filtering off whey | Grams of zinc lactate by |                | Hours of extraction with wet method |
|-------------|---|--------------------------|----------------|-------------------------------------|
|             |   | dry extraction           | wet extraction |                                     |
| A4          | —   | 4.3037                   | 2.4142         | 48                                  |
| A5          | —   | 1.7871                   | 1.1655         | 48                                  |
| A6          | +   | 1.1614                   | .2634          | 72                                  |
| S6          | +   | 2.3196                   | .8317          | 48                                  |
| S7          | +   | 3.7601                   | .7961          | 48                                  |
| S8C         | +   | 4.7479                   | 5.1395         | 72                                  |

paratively high in one instance. It seems probable that the amount of acid produced by the culture is a more important factor here than the use of  $H_2SO_4$  in securing the whey.

The lower yield with the wet extraction method may explain the differences in the moisture contents of the lactates prepared by the two methods since where there was a considerable portion of the acid left unextracted it would seem that the inactive and active acids might not have been removed in proportional amounts.

#### Discussion of Results

The twelve cultures that were studied on the assumption that they were *L. acidophilus* showed variations in a number of characters. The character of chief concern in this investigation was the isomeric form of lactic acid produced and in this the cultures varied from practically pure active to practically pure inactive acid; with the active acids the rotation was dextro in all cases. There were four cultures which gave inactive or largely inactive acid and these had a number of characters which seemed to be correlated with this. They all grew at  $45^\circ C.$  and none of them at  $15-20^\circ C.$ , all failed to grow at a low surface tension with the medium em-

ployed and all were comparatively large; two of the other eight cultures resembled them in failing to grow at a low surface tension and two resembled them in size. One of the four cultures did not ferment maltose although all the other cultures did.

The seven cultures which were isolated from the feces of an infant, an adult, a calf or a rat and one of the cultures from a commercial laboratory were quite constant in the characters studied; all of them produced largely or entirely dextro lactic acid, all grew at 15-20°C. but not at 45°C., six of the eight grew at a low surface tension with the medium used and all fermented maltose. This uniformity in the isolated cultures is interesting because all were isolated from fecal material.

The general results obtained suggest that the identification of *L. acidophilus* on the basis of the tests now commonly used is not an easy matter and also that there is no uniform understanding as to the characters of this organism. There is of course the possibility that the four cultures that are rather outstanding in their characters, some of which are different than the characters usually ascribed to *L. acidophilus*, should not be classed as *L. acidophilus* at all but as one of the closely related organisms, perhaps *L. bulgaricus*. If this is not advisable the organisms studied as *L. acidophilus* should be divided into two or more types. Which of these steps is preferable depends on the results of further studies of the group of organisms to which *L. acidophilus* belongs.

### CONCLUSIONS

1. The isomeric form of lactic acid produced by the twelve cultures of so-called *Lactobacillus acidophilus* varied from practically pure active to practically pure inactive acid; with the active acids the rotation was dextro in all cases.
2. Variations were also encountered among the cultures studied in the characters which are used in identifying *L. acidophilus*.
3. There was a tendency toward a correlation of characters with the production in milk of lactic acid that was largely or entirely inactive; these included growth at 45°C. but not at 15-20°C., failure to grow at a reduced surface tension with the medium employed and a comparatively large size.
4. The results suggest that the organisms studied as *L. acidophilus* should not all be classed together. Some of them should either be grouped with one of the closely related organisms such as *L. bulgaricus* or *L. acidophilus* should be divided into two or possibly even more types.

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     *saturnus*, 339.  
 Yeast growth stimulant, 411.  
*Zygodesmus*, 340.  
*Zygorhynchus*, 227, 251.  
     *heterogamus*, 252.  
     *moelleri*, 32, 252.  
     *vuilleminii*, 28, 29, 32, 252.











